2

Total Recycle System of Food Waste for Poly-L-Lactic Acid Output

Kenji Sakai[1], Pramod Poudel[1,2] and Yoshihito Shirai[3]
[1]*Graduate School of Bioresource and Bioenvironmental Sciences, Faculty of Agriculture, Kyushu University, Fukuoka,*
[2]*National College (NIST), Department of Microbiology, Tribhuvan University, Kathmandu,*
[3]*Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu, Fukuoka,*
[1,3]*Japan*
[2]*Nepal*

1. Introduction

1.1 Impacts of food waste

Food waste is defined as wholesome edible material intended for human consumption arising at any point in the food supply chain that is instead discarded, lost, degraded or consumed by pests. The average consumer in Europe and North-America throws away ca. 100 kg of food per year according to a new report published by the UN's Food and Agriculture Organization (Annual report of FAO, 2011 & Parfitt et al., 2010). The study centers on food loss and food waste during the whole supply chain from production to consumption and finds that around "one-third of the edible parts of food produced for human consumption gets lost or wasted globally" representing about 1.3 billion ton per year. Around 20% of about 50 metric tons of waste that is generated annually in Japan is high moisture content refuse from kitchens and the food industries. The social, economic and environmental impacts of food waste are enormous. Such wastes readily decompose, generate odors, and sometimes cause illnesses. Municipal solid wastes including food waste are usually incinerated or land filled which ultimately generates many problems such as liberation of harmful compounds like dioxin and furans (Addink & Olie, 1995). Incineration facilities can be damaged by temperature fluctuations when food waste with high water content is burned in semi continuous process. In addition, it is difficult to recover energy from such waste incineration processes because the heating value of food waste is low (Harrison et al., 2000). This requires frequent and periodic collection and treatment of waste i.e. irrespective of their values. When excess food waste is disposed of in a landfill, it decomposes and is a significant source of methane gas, which is highly effective at trapping heat in the atmosphere than CO_2 (Camobreco et al., 1999). Annually, food waste in the United States accounted for slightly more than 100 metric tons of methane originating from landfills. At the European level, the overall environmental impact is at least 170 metric tons of CO_2 emitted annually.

In this regard, the significance is considered as an important concept for aiming at the formation of the recycle-oriented society. Accordingly, untreated food waste contributes to

excess consumption of freshwater and fossil fuels which, along with methane and CO_2 emissions from decomposing food, impacts global climate change. The prompt implementation of total recycling system can play a beneficial role in the utilization of municipal waste.

The design of this system can be conducted considering not only to the environmental impacts and energy increase in the recycling but also to the best economical efficiency as sustainable bio-based materials. So, management of municipal solid waste including kitchen waste via microbiological processes improves these wastes and reduces the need for both landfill space and fuel used in waste incineration. Direct composting and methane fermentation, which produce fertilizers and biogas, are alternative ways to reuse food waste but these processes have been applied only in rural areas. On the other hand, it has been found that municipal food waste is nutritious substratum for natural lactic acid bacteria (Sakai et al., 2000b). This finding indicated another reuse route of food waste, suitable for urban areas.

	No.	Amount/Place [Kg/y]	Total Amount [t/y]	Impurity [%]	Food waste [t/y]	Total sugar [t/y]	Glucose [t/y]
Large scale retail store (1)	28	437.5	12.3	15.6	10.4	1.5	1.3
Large scale retail store (2)	124	290.0	36.0	13.1	31.3	2.2	1.8
Convenience store	299	17.6	5.3	8.2	4.9	1.6	1.1
Hotel (>100 room)	29	160.0	4.6	1.8	4.5	0.5	0.4
Hospital (>100 bed)	64	143.5	9.2	0	9.2	1.1	0.8
Department	10	542.7	5.4	12.5	4.7	0.5	0.5
Total			72.8		65.0	7.4	5.9

Table 1. Food waste recycling generated in Kitakyushu-City, Japan.

According to the report of Shirai & Sakai (2006), food waste collected from each town sectors of Kitakyushu-city of Japan, as shown in Table 1 above, accumulated 7.4 tons of sugar per year (7.4 t/y) out of which consisted 80% glucose after the treatment with very low concentration of enzyme. Further, the overall glucose generated from the food waste from house kitchen is shown in Table 2 below.

Day	Site	Food composition (%)				Water (%)	Total Sugar (g/kg wet waste)	Glucose (g/kg wet waste)
		Cereal	Fish & Meat	Vegetables	Fruits			
2003/1/21	1	17.6	10.5	55.5	16.4	76.2	115	89
2003/1/21	2	21.5	9.1	32.7	36.7	75.3	131	78
2003/1/21	3	10.0	13.7	76.3	0	72.7	93	64
2003/1/22	4	15.8	8.4	42.5	33.3	75.9	108	104
2003/1/22	5	12.4	5.1	37.9	44.6	76.5	120	76
2003/1/24	1	24.0	12.6	23.1	40.3	76.5	148	71
2003/1/24	2	20.4	28.0	30.6	21.0	80.5	108	55
2003/1/24	3	11.1	11.0	57.2	20.7	73.8	139	86
2003/1/25	4	11.0	11.0	57.4	20.5	75.2	139	77
2003/1/25	5	13.0	6.4	47.2	33.4	72.4	191	117
Average		15.7	11.6	46.0	25.1	75.5	129	83

Table 2. Composition of food refuse wasted from house kitchen at Kitakyushu area.

2. Bio-economy system

Today's industrial economy is largely dependent on petroleum oil which provides the basis of most of our energy and chemical feedstock. There is increasing concern over the impact of these traditional manufacturing processes on the environment. Therefore, considering to the resource materials' exhaustion, we need to substantially reduce our dependence in the petroleum feedstock by establishing a bio-based economy.

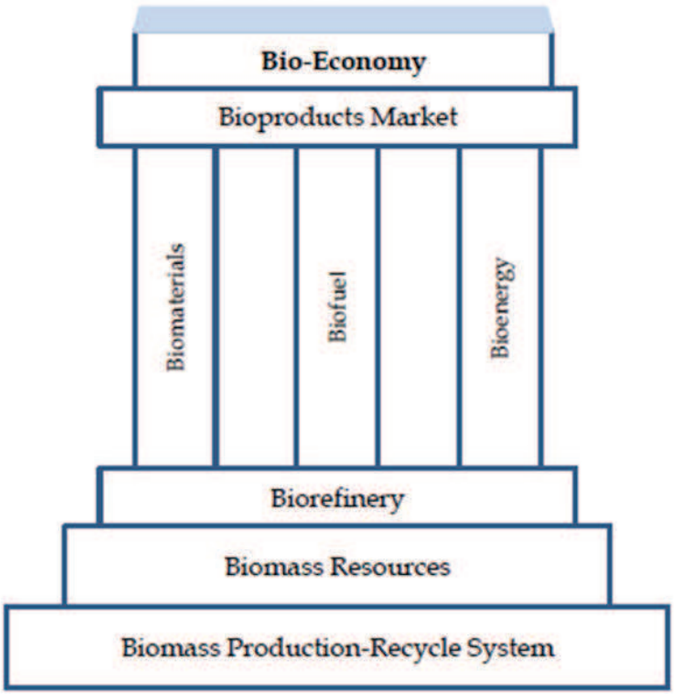

Fig. 1. Schematic Presentation of Sustainable Bio-Economy System (Revised from Kamm et al., 2005).

The bio-economy is the sustainable production and conversion of biomass for a range of food, pharmaceuticals, fiber and industrial products, and energy. In it, the renewable biomass encompasses any biological material to be used as raw material (KBBE, 2010). It helps to:

increase the scientific understanding of biomass resources and improve the tailoring of those resources; improve sustainable systems to develop, harvest and process biomass resources; improve efficiency and performance in conversion and distribution processes and technologies for the development of bio-based products; create the regulatory and market environment necessary for sustainable development and the use of bio-based products (Fig. 1). Bio-based products are virtually similar to their petroleum-based counterparts but they are manufactured from renewable resources (Kamm et al., 2005).

Generally biomass resources are strategic plant biomass rich in sugar (corn, rice, cassava, cane, beet etc) and oil (oil palm). To establish bio-economy, it is prerequisite to avoid confliction with food, cultivation field and deforestation. There are also important biomass resources in residues from agriculture and forestry including both wet and dry waste

materials, for instance, sewage sludge and municipal solid waste. One of the pillars of bio-economy system is the systematic conversion of biomass resources i.e. bio-refinery (Fig. 1). Similarly, the increased production of bio-fuels, especially biodiesel from the transesterification of fats and oils from oil plants (Palm, Soybean, Jatropha etc), is making glycerin a cheap organic material. Basically, conversion of these biomass resources to useful sustainable products includes two general pathways: thermo-chemical and bio-chemical conversion pathways. Briefly, biochemical conversion pathways use microorganisms to convert biomass resources into methane, hydrogen gas, and organic acid or simple alcohols usually in combination with some mechanical or chemical pre-treatment step. Substantial research effort has been expended to make this a raw material for various organic chemicals. Not the least of these is material that can be used in various thermoplastic and thermo-set polymers. Equally important, succinic acid, a biomass derived product posits its large potential for a variety of applications. This dicarboxylic acid can be converted to a huge amount of green chemical of industrial value, such as polyesters (derived from succinic acid and butandiol) which is used for soft plastics.

3. Poly-L-lactic acid

3.1 Lactic acid

Lactic acid has both hydroxyl and carboxyl groups with one chiral carbon atom existing in two stereoisomers L- and D-lactic acid, and it is widely used in the food, pharmaceutical, and general chemical industries (Sakai et al., 2001). The L form differs from the D form in its effect on polarized light. For L-lactic acid, the plane is rotated in a clockwise (dextro) direction; whereas, the D form rotates the plane in an anticlockwise (laevo) direction. Basically, the chemical synthesis only produces the racemic mixture of the L (+) and D (-) enantiomers, while microbial fermentation using biomass resources has the advantage of producing optically pure L(+)- or D(-)-lactic acid (Hafvendahl & Hagerdal, 2000). Among basic compounds from biomass, lactic acid is relatively unique C-3 compound obtained from C-6 glucose without any oxidation-reduction of the carbon atoms. Lactic acid can be polymerized to form the biodegradable and recyclable polyester poly-lactic acid which is considered a potential substitute for plastics manufactured from petroleum (Ohara & Sawa, 1994). No doubt, we are subsequently focusing on the production of lactic acid with high optical purity from food waste (kitchen refuse).

Optical purity is measured as;

$$\text{Optical purity } (\%) = ([L] - [D]) \times 100 / ([L] + [D])$$

where [L] denotes to the concentration of L-lactic acid and [D] to that of D-lactic acid.

We found that food waste collected from commercial sectors such as retail store, convenience store, college and university contained high amount of total sugars (129g/kg) as shown in Table 1 and 2 (Shirai & Sakai, 2006). They are mainly starch and can easily be converted to glucose enzymatically (82g/kg). Subsequently, for the production of lactic acid generating glucose from the food refuse was subjected to the fermentation using *Lactobacillus rhamnosus* which produces high amount of L-lactic acid (Sakai et al., 2004a, Fig.2). Considerably, the rate of lactic acid production was more than 85% which was satisfactory with the highest yield.

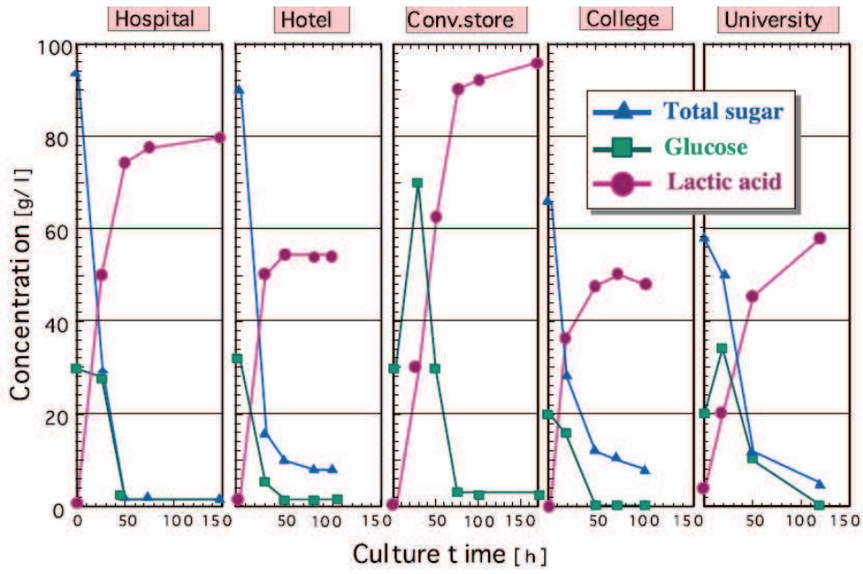

Fig. 2. Lactic acid production profile from different commodities

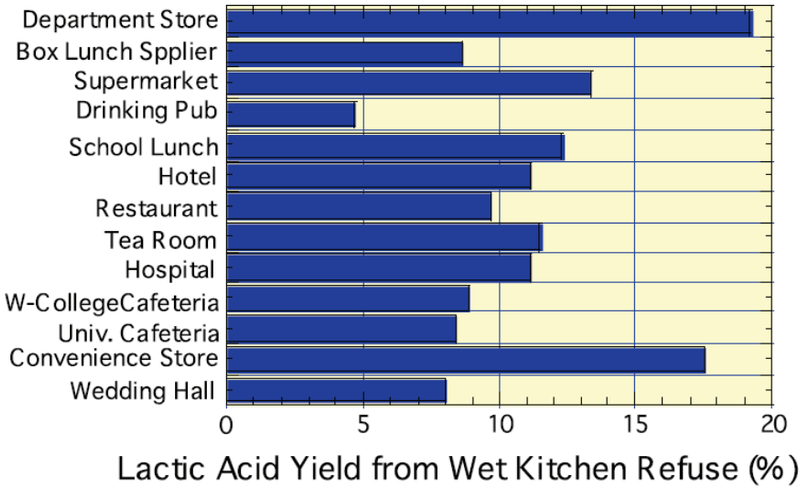

Fig. 3. Lactic acid yield using various kitchen refuse from commercial sectors

3.2 Poly-lactic acid from food waste

Poly lactic acid (PLA) is thermoplastic aliphatic polyester synthesized from L- or D-Lactic acid (Fig.4). Highly optical pure L- or D-lactic acid is necessary to obtain high crystalline poly-lactic acid which leads to the high strength, chemical and heat resistances of the polymers. PLA polymers range from amorphous glassy polymers with a glass transition of 58°C to semi-crystalline/ highly crystalline products with crystalline melting points ranging from 130°C to 180°C.

We propose a novel recycling system for municipal food waste that combines fermentation and chemical processes to produce high-quality poly-L-lactate (PLLA) biodegradable plastics (Fig. 5). The process consists of removal of endogenous D- or L-lactic acid from minced food waste by a *Propionibacterium*, L-lactic acid fermentation under semisolid

conditions, L-lactic acid purification via butyl esterification, and L-lactic acid polymerization via LL-lactide. The total design of the process enables a high yield of PLLA with high optical activity (i.e., a high proportion of optical isomers) and novel recycling of all materials produced at each step with energy savings and minimal emissions. Approximately, 50% of the total carbon was removed mostly as L-lactic acid and 100 kg of collected food waste yielded 7.0 kg PLLA. The physical properties of the PLLA yielded in this manner were comparable to those of PLLA generated from commercially available L-lactic acid (Table 4). Evaluation of the process is also discussed from the viewpoints of material and energy balances and environmental impacts (Fig. 5).

Although the ester bond of poly-L-lactate (PLLA) is susceptible to some enzymes, including proteinases and lipases, and PLLA has been recognized as a biodegradable plastic (Sakai et al., 2001), its biodegradation in soil is rather slow and it depends on morphology and thickness (Miyazaki & Harano, 2001). Therefore, PLLA may better be developed as a chemically recyclable plastic with an appropriate collection system for the used materials but not as a single-use plastic (Nishida et al., 2004).

Monomers and Dimers	Melting Point (°C)	Poly-lactic acid	Melting point (°C)
L-lactic acid	25.8	PLLA/PDLA Stereo-complex	245
DL-lactic acid	16.8	PLLA	245
LL-lactide	97.8	PLLA	175
Meso-lactide	52.0	PDLA	175
DL-lactide (Co-crystal)	124.0	PLDLA	50

Table 3. Melting point for Lactic acid and its Polymers

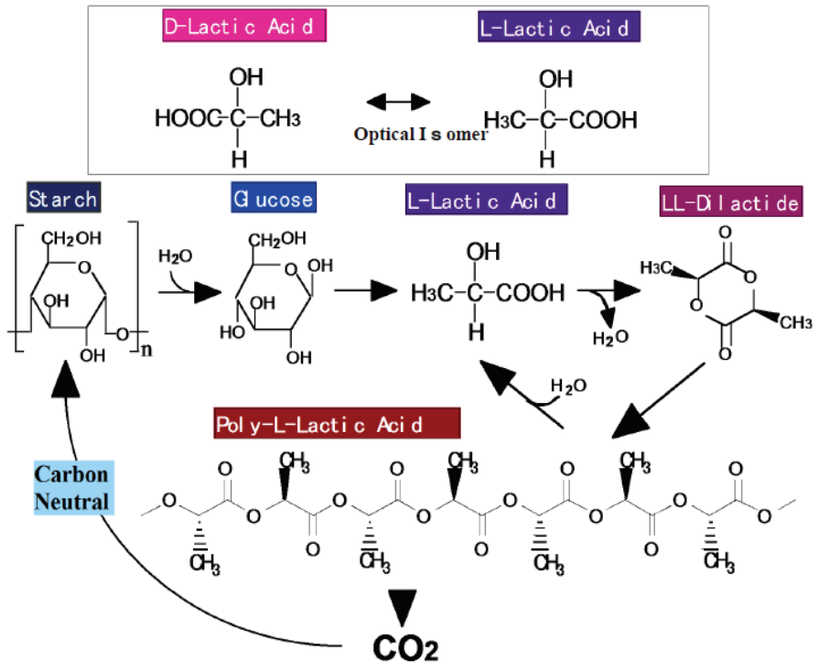

Fig. 4. Poly- L-lactic acid from starch

The proposed PLLA process has an energy advantage over even the general poly-lactide process because the feedstock is totally food waste. In the process with corn starch, nearly 30% of gross fossil energy use goes into producing and processing corn to provide dextrose to feed the lactic acid fermentation. Since the feedstock to the proposed PLLA process is food waste that must otherwise be disposed of, the only upstream fossil energy allocated to the production of PLLA would be that required for collection of the separated waste (Sakai, 2004b, 2007).

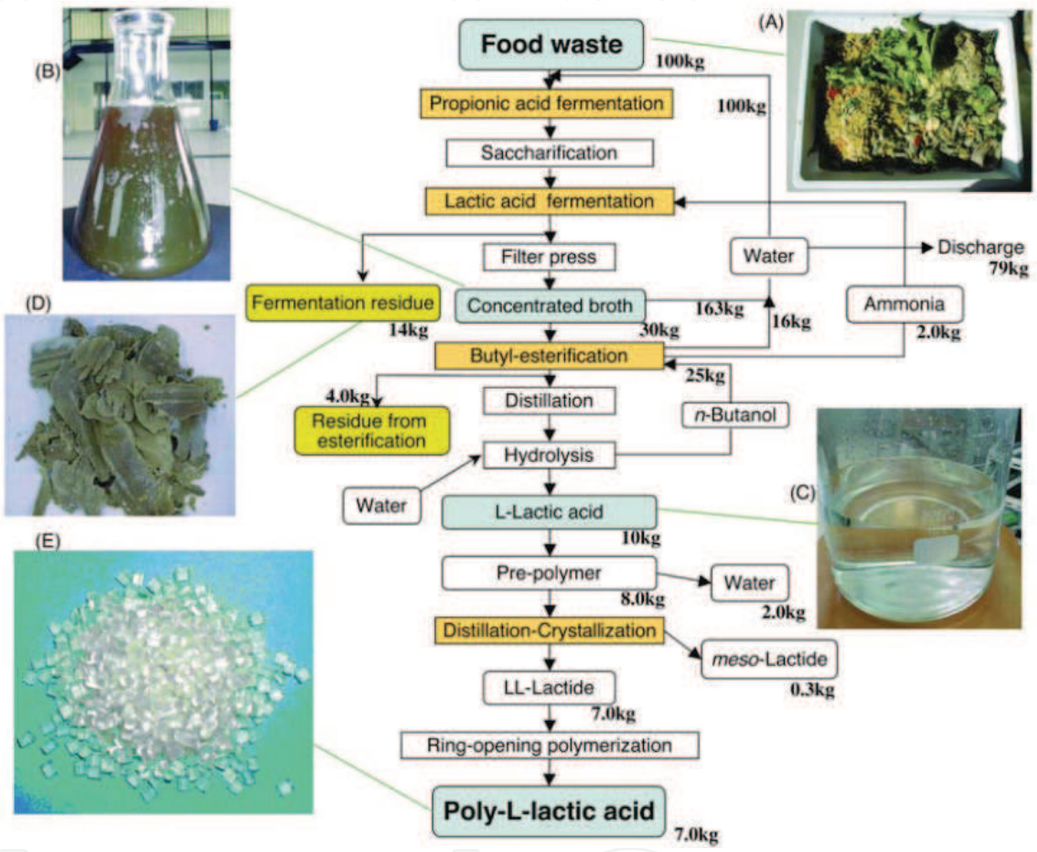

Fig. 5. Process outline of PLLA production from food waste (A), Food waste (B), concentrated broth after lactic acid fermentation (C), Purified L-lactic acid (D), Fermentation residue (E), Pellets of PLLA.

Optical purity	97.5%
Average molecular weight	200kDa
Melting point	175°C
Glass transition temperature	58°C

Table 4. Characteristics of PLLA produced from collected food waste.

The material balance and energy requirements of the total process are summarized in Table 5. The overall experimental process yielded 68.8 g PLLA from 1 kg food waste (1.0 kg PLLA/14.6 kg food waste). This means that 34% of total carbon in the food waste was recovered as PLLA. In comparison, the first commercial PLLA plant operated by Cargill Dow Polymers reportedly requires gross fossil process energy of 39.5 MJ/kg (Vink et al., 2003). Meanwhile, the process

energy required for production of bottle grade polyethylene terephthalate (PET) and high-density polyethylene (HDPE) using petrochemicals is 27 MJ/kg and 23 MJ/kg respectively (Boustead, 2002). Furthermore, the process was designed to have low environmental impact. The fermentation residue is rich in nitrogen (C/N=6.5; concentrations of N, P and K were 75, 2.6, and 0.7 mg/g dry matter respectively) reduced in weight to 14% of the untreated food waste, and the precipitated residue produced at the esterification step contains high concentrations of phosphorus and potassium (C/N = 7.7; concentrations of N, P and K were 39, 28, and 23 mg/g dry matter respectively). These stable residues were confirmed to be useful fertilizers (Mori et al., 2008). Condensed water, ammonia, and butanol were reused during the process. Consequently, nearly all materials are converted to valuable resources or recycled in the process. As the production energy required is comparable to that required in the PLLA process using maize, we have been trying to improve the process especially to reduce energy required at the process of lactic acid fermentation as described below.

Besides recycling process of municipal food waste using mesophile (*L. rhamnosus*), the prompt utilization of its biomass as a feed additive for the animals was also proceeded to fulfill the zero emission concept (Umeki, 2004, 2005).

Items	Content and Yield		Carbon yield (%)
	(Kg/Kg wet waste)	(Kg/Kg dry waste)	
Dry material[a]	0.215	1.0	-
Carbon content[a]	0.101	0.470	100
Total soluble sugar[a,b]	0.143	0.665	-
Lactic acid in culture filtrate[c]	0.118	0.549	47
Purified L-lactc acid[c]	0.099	0.459	37
PLLA[c]	0.069	0.320	34
Fermentation residue[d]	0.14	0.101	27
Esterification residue[e]	0.04	0.038	16

[a] Average of 20 samples from 15 companies.
[b] Average concentration in saccharified samples.
[c] PLLA was experimentally produced from three representative culture filtrate samples. Average yield was calculated using efficiencies of each step (purified L-lactic acid from culture filtrate, 78.7%; PLLA from purified L-lactic acid, 91.9%).
[d] Representative data: water contents of fermentation residue and esterification residue were 38% and 6.4%, respectively.

Table 5. Product yield and carbon balance

4. Microorganisms for lactic acid production (MLAP)

4.1 Lactic acid bacteria (LAB)

The term lactic acid bacteria (LAB) means bacterial group that produces lactic acid as the major metabolite, and is used in different meaning from microorganism for lactic acid production (MLAP): they are gram-positive, acid tolerant, non-sporulating, non-respiring rod or cocci with low-GC content, able to produce L-type, D-type, or, L/D lactic acid as the major metabolic end product (more than 50%). Maximum growth temperature of it is up to 43°C. The core genera of LAB are *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus,* and *Streptococcus* as well as the more peripheral *Aerococcus*, *Carnobacterium*, *Enterococcus,*

Oenococcus, Teragenococcus, Vagococcus, and *Weisella* belonging to the order *Lactobacillales*. *Lactobacillus rhamnosus* has been reported for L-lactic production from kitchen refuse (Sakai et al., 2004b). Similarly, Oh et al., (2005) used strains of *Enterococcus faecalis* for the lactic acid production from sterilized wheat hydrolysates. On the other hand, microorganism which produces high amount L-lactic acid and is used for industrial lactic acid production (MLAP) distributed in more variety of genera in bacteria, yeast, and fungi.

4.2 Non-LAB

As non-LAB, *Rhizopus oryzae*, *R. microsporus*, *Bacillus subtilis*, *B. coagulans* has been used for L-lactic acid production (Miura et al., 2003, Ohara et al., 1996 & Sakai et al., 2006c). Particularly, optically active L-lactic acid production from *Rhizopus oryzae* strains is significant (Miura et al., 2003). Industrial production of L-lactic acid using *Rhizopus* sps. has several advantages over using lactic acid bacteria (LAB).

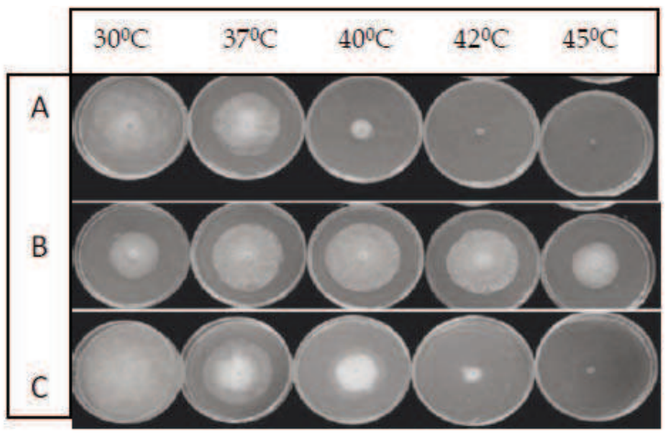

Fig. 6. Effect of incubation temperature on the growth of isolates. A) *Rhizopus oryzae* (TISTR 3514), B) *Rhizopus microsporus* (TISTR 3518) and C) *Rhizopus oryzae* (TISTR 3523).

The fungus only produces L-lactic acid, while LAB frequently produces the D-isomers as well. Therefore, the optical purity of L-lactic acid produced from the fungus is relatively higher than that from LAB. L-lactic acid production has been reported in only the *R. oryzae* group. In addition, variety of studies on construction of lactic acid-producing *Escherichia coli* and *Saccharomyces cerevisiae* by genetic engineering have been reported (Sakai, 2008).These strains would be promising for the industrial production under strictly closed sterilized fermentation using certain purified substrate sugar. According to Kitpreechavanich et al., (2008), a thermotolerant *Rhizopus* strain which is capable of producing L-lactic acid from starch substrate was identified as *R. microsporus* (Fig. 6).

4.3 Thermophilic/thermotolerant bacteria MLAP

The term 'thermophilic' has been progressively more restricted to organisms which can grow or form products at temperatures between 45°C and 70°C with optimal 60°C (Madison et al., 2009). Dijkhuizen & Arfan (1990), reported that thermotolerant organisms grow at temperature between 35°C and 60°C with optimal 50°C -55°C. Thermophilic bacteria are common in soil, compost and volcanic habitats and have a limited species composition (Zeikus, 1979).

Meantime, we have found that several thermotolerant/thermophilic bacterial species in Bacillaceae are able to produce certain amount of optically active L-lactic acid (Table 7). Compared to *Lactobacilli* and *Lactococci*; *Bacillus* species generally show interesting microbial properties. Most of them are basically aerobic and they form spores under certain environmental conditions. They do not produce D-lactic acid. Some of them show growth limitation at temperatures around 70°C. Some species produce polysaccharide-hydrolyzing enzymes such as amylase, chitinase, or xylanase. Many strains ferment glycerol, D-galactose, D-fructose, D-xylose, sucrose, cellobiose as well as starch which are constituent sugars in food and agricultural waste. Therefore, not only the characteristics of this bacterium are quite suitable for the bioconversion of starch from food waste but also it would be applicable to other agricultural wastes.

Thermotolerant strain *Bacillus licheniformis* has been explored for the L-lactic acid production from standard kitchen refuse under open condition i.e. 40g/*l* L-lactic acid with 97% optical activity and 2.5g/*l*.h productivity (Sakai & Yamanami 2006b). Moreover, thermophilic bacterium *Bacillus coagulans* is quite useful for producing optically active L-lactic acid from non-steriled kitchen refuse (Sakai & Ezaki, 2006c). The *B. coagulans* selectively grew at 55°C under open condition, while *Lactobacillus plantarum*, which is a major species in natural fermentation of kitchen refuse under mesophilic condition, suppressed its growth. Temperature and growth relations in different temperature classes of *B. coagulans* and *L. plantarum* are shown in Fig. 7.

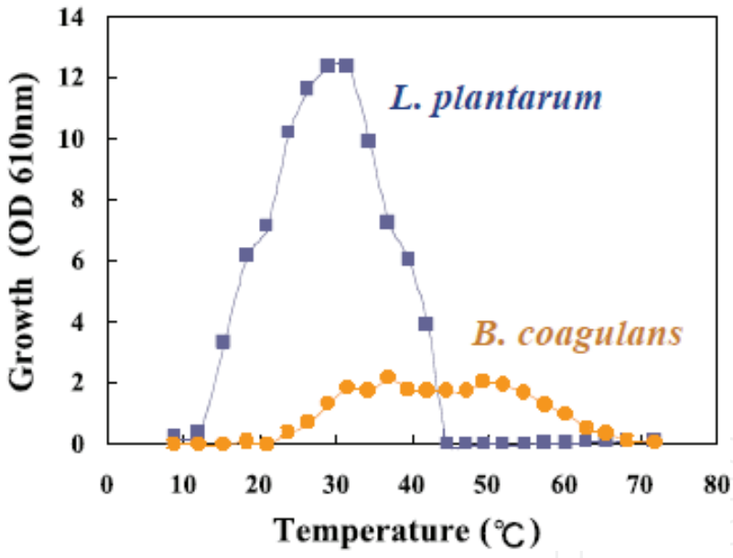

Fig. 7. Effect of temperature on growth of *B. coagulans* and *L. plantarum*

5. Open fermentation for total recycle of food waste

5.1 Merits of open fermentation

Nonsterile open fermentation has various merits over conventional sterilized and closed fermentations. For example, it requires no facilities for sterilization and no steam for autoclaving. Thus, nonsterile open fermentation of kitchen refuse could be implemented at on-site storage facilities for municipal food waste before the waste is transported to centralized processing plants. Because autoclaving is avoided, substrate sugars and other nutritional

constituents required for lactic acid fermentation remain intact. The Maillard reaction, for instance, not only decreases the amount of available sugars and amino acids but also produces unfavorable furfural compounds that inhibit bacterial growth. In addition, food waste is unsuitable for filter sterilization or separate autoclaving of substrate from other medium constituents. Nonsterile open fermentation avoids these complications; however, the optical purity of accumulated lactic acid from such fermentation at room temperature is low (Sakai & Ezaki, 2006c). This type of natural lactic acid fermentation also occurs during the collection and storage of municipal kitchen refuses (Sakai et al., 2004b). On the other hand, the thermophilic bacterium *Bacillus coagulans* is useful for producing optically active L-lactic acid from kitchen refuse under nonsterile condition (Heriban et al., 1993).

5.2 Mesothermal recycle of food waste

During the investigation of open fermentation at atmospheric temperature, we found that naturally-existed mesophile *Lactobacillus plantarum* preferentially proliferated and selectively accumulated lactic acid in non-sterile kitchen refuse (food waste) under pH swing control (intermittent pH adjustment) (Table 6). Despite the reproducible and selective proliferation of the species, this strain produced both L- and D-lactic acid with nearly equal racemic body ratio. As optically inactive lactic acid is not suitable for high-quality of PLA, we tried to improve the optical activity by inoculating *L. rhamnosus* or *Lactococcus lactis* which are L-lactic acid producing LAB. But this kind of open fermentation also resulted proliferation of naturally existed *L. plantarum* and accumulation of lactic acid with low optical activity. In comparison, Frederico et al., (1994) also reported that *L. plantarum* accumulated low amount of lactic acid during the fermentation of fruit juice under sterilized condition. As shown in Table 6 (Run 1-1 to 1-5), the amount of accumulated lactic acid varied according to the intervals of pH adjustment, and maximum accumulation was observed with pH adjustment of 6hour (6h) or 12h.

Run[a)]	Adjusted pH	Interval (hour)[b)]	Productivity (g/*l*.h)[c)]	Accumulation (g/*l*)[d)]	Selectivity (%)[e)]
1-1	7	0	1.05	19	83
1-2	7	6	0.70	44	92
1-3	7	12	0.58	45	94
1-4	7	24	0.4	31	94
1-5	7	_[f)]	0.25	13	87
2-6	3	_[g)]	0.04	2.0	-
2-7	5	6	0.42	32	96
2-8	7	6	0.65	45	94
2-9	10	6	0.58	45	92

[a)] MKR samples of runs 1-1 to 1-5 and runs 2-6 to 2-9 were differently prepared.
[b)] Interval of intermittent pH adjustment.
[c)] Average production rate of lactic acid to reach maximum concentration.
[d)] Maximum concentration of lactic acid accumulated.
[e)] Ratio of accumulation of lactic acid to total organic acids
[f)] MKR paste was adjusted at pH 7.0 initially and incubated without pH adjustment.
[g)] No pH change was observed

Table 6. Effect of intermittent pH adjustment on accumulation of lactic acid during the open fermentation of MKR paste by mesophile.

5.3 Molecular monitoring of bacteria during recycle of food waste

From the very nature of a thing, non-sterilized fermentation process generally proceeds under a mixed culture condition. We have repeatedly isolated and identified the microbial structure during the course of open fermentation of kitchen refuse. Meantime, we cultivated, purified and characterized several microbial isolates, which counts laborious and time-consuming, and only predominant cultivable species can be identified. Therefore, we applied 16SrRNA-targeted fluorescence *in-situ* hybridization (FISH) to analyze the microbial population during open lactic acid fermentation (Sakai et al., 2004a, Fig. 8). For this, we designed probes for monitoring non-sterilized open fermentation of kitchen refuse such as a LAB group specific probe (LAC722) and a *B. coagulans* specific probe (Bcoa191). Similarly, specificity of Bcoa191 probe for *B. coagulans* in whole-cell hybridization of the new probe was confirmed *B. coagulans*, and differentiated the species from other bacteria as shown is Fig. 9 (Sakai & Ezaki, 2006c).

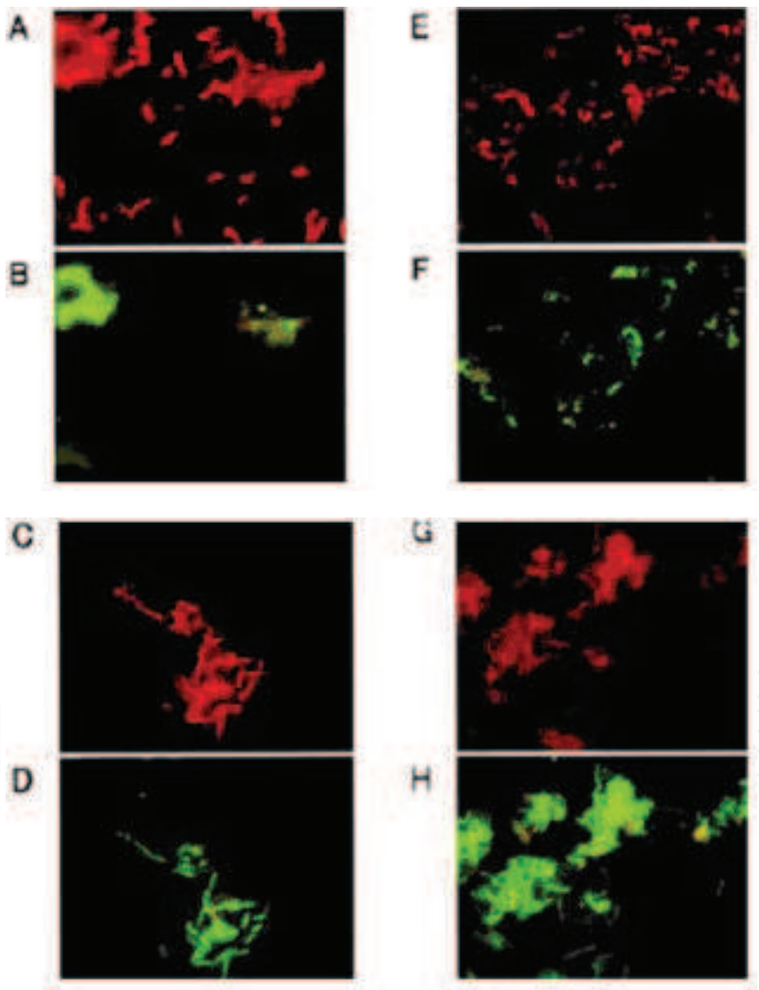

Fig. 8. Typical FISH staining during open fermentation of kitchen refuse. Samples at time zero (A, B, C, D), or 48 hours (C, D, G, H), without (A, B, C, D) or with (E, F, G, H) inoculated seed culture stained with rhodamine-EUB338 (A, C, E, G) or FITC-LAC722(L) (B, D, F, H), (Sakai et al., 2004a).

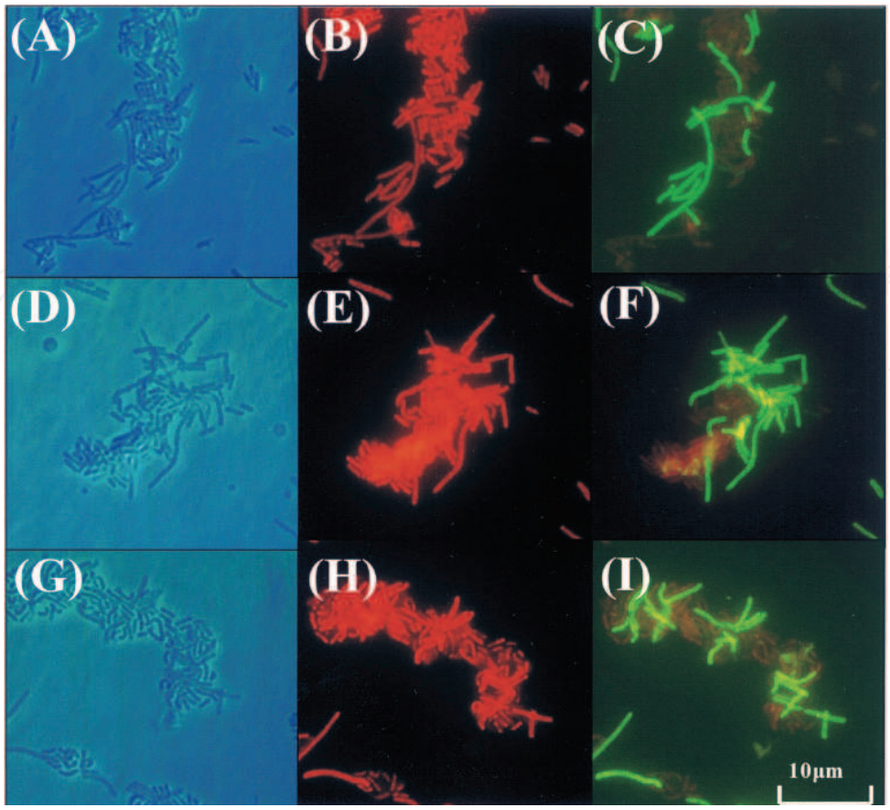

Fig. 9. Differential staining of *B. coagulans* using new probe Bcoa191 in 16S-Fluorescence In Situ Hybridization (FISH). *B. coagulans* cells were mixed with *L. plantarum* (A-C), L. *rhamnosus* (D-F), or E. *coli* (G-I). The mixed-cell samples were subjected to 16S-FISH, and the photomicrographs of phase contrast microscopic observation (A, D, G) and fluoro-microscopic observation for rhodamine-EUB338 (B, E, H) or FITC-Bcoa191 (C, F, I) are shown (Sakai & Ezaki, 2006c).

5.4 Thermotolerant MLAP in total recycle of food waste

As shown in Table 6, the L-lactic production rate and optical purity of mesophilic lactic acid bacteria was low. We, furthermore, tried to use the thermotolerant *Bacillus* species for the total utilization of food waste for PLA production and its biomass utilization. Production of lactic acid by some *Bacillus* species, including *Bacillus coagulans*, *Bacillus stearothermophilus*, *Bacillus subtilis* and *Bacillus licheniformis*, had already been reported (Bischoff et al., 2010, Heriban et al., 1993; Ohara & Yahata, 1996; Sakai & Yamanami, 2006b).

Recently, we isolated and identified novel thermotolerant *Bacillus* species from the mixed culture system. We, subsequently, used these strains for L-lactic acid production from the food waste. During the total utilization of food waste, the conditions for the fermentation of food waste were optimized as described previously (Sakai, 2006a, 2006e). Interestingly, novel thermotolerant strains *B. soli* U4-3 & U4-6 and *B. subtilis* N3-9 produced high amount of L-lactic acid within 6 hours of fermentation at 50°C with cent percent optical purity. L-lactic acid production profile is shown in Table 7 below. Meantime, L-lactic acid produced was further used for the PLA production which is one of the instances in total recycle of food waste.

Isolate No.	Species	L-lactic acid (g/*l*)	Yield/g (%)	Optical Activity (%)
N1-3	*Bacillus coagulans*	20.6	69.0	99.8
N1-4	*B. coagulans*	25.1	61.1	99.9
N1-7	*B. niacini*	11.7	46.4	99.5
N1-12	*B. coagulans*	28.6	60.2	100
N2-1	*B. coagulans*	29.1	64.0	99.4
N2-10	*B. subtilis*	31.5	68.9	99.0
N3-6	*B. subtilis*	32.6	82.7	99.7
N3-9	*B. subtilis*	35.1	74.0	100
U4-3	*B. soli*	30.3	70.8	100
U4-6	*B. soli*	29.3	85.5	100
N5-7	*B. subtilis*	28.4	61.0	99.3
N5-8	*B. subtilis*	23.3	55.3	99.0

Table 7. L-lactic acid production by thermotolerant *Bacillus* strains isolated from high-temperature and Aerobic fermenter.

In general, for the commercial production of poly-L-lactic acid plastic from biomass wastes, a feasible fermentation process to produce optically active L-lactic acid is required (Sakai, 2004a, 2004b, 2006d). By using collected kitchen refuse, saccharified liquid containing 117g/*l* soluble sugar was obtained (Table 8). This figure is fairly representative of collected kitchen refuse (Table 2). Following the incubation with B. *coagulans* at 55°C, pH 6.5, 86g/*l* L-lactic acid with 97% optical purity was produced under non-sterile conditions. The yields of total lactic acid from total carbon and total sugar were 53% and 98% respectively. These figures are comparable to those achieved by *L. rhamnosus* incubation using sterilized collected kitchen refuse (Fig.10).

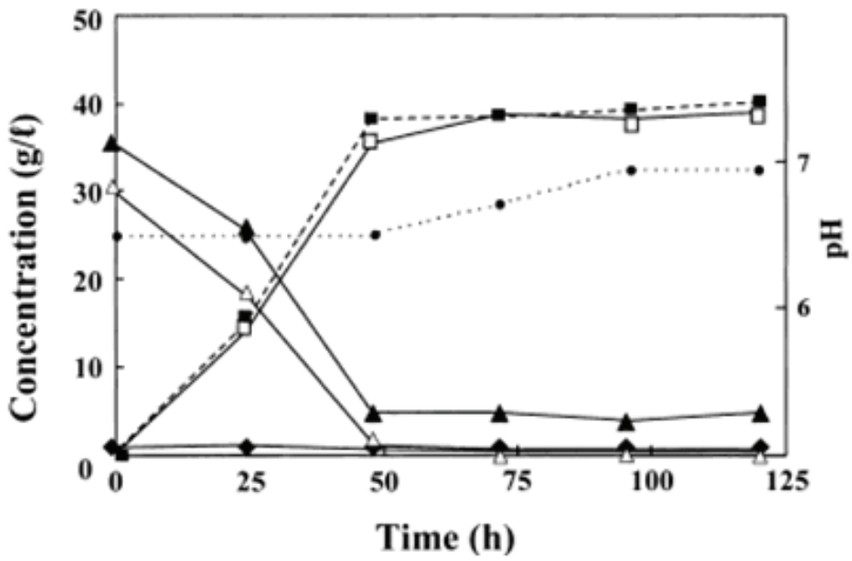

Fig. 10. Open fermentation of MKR using *B.* coagulans under constant pH 6.5 at 55°C under open culture conditions. The changes in the concentrations of total lactic acid (closed squares), L-lactic acid (open squares), D-lactic acid (closed diamonds), total sugar (closed triangles), and glucose (open triangles) are represented along with pH change (close circles).

Parameters	Closed fermentation with *Lactobacillus rhamnosus*		Open fermentation with *Bacillus coagulans*	
	Initial	Final	Initial	Final
Total sugar (g/*l*)	74	14	117	31
Total lactic acid (g/*l*)	3	61	2	86
Optical purity (%)	1.4	95	2.9	97
Carbon yield (%)	-	38	-	53
Sugar yield (%)	-	97	-	98

Table 8. Summary of open and closed fermentation of kitchen refuse using mesophile and thermophile.

6. Conclusions and future prospective

The majority of the worldwide industrial economics are now largely dependent on petroleum oil which provide basis for most all of our energy and chemical feedstock. Meanwhile, there is increasingly concern over the impact of these traditional manufacturing processes or the environment, i.e. the effect of CO_2 emissions on global warming as well as exhaustion of fossil resources. In order to maintain the world population in terms of food, fuel, and organic chemicals, we need to substantially reduce our dependence on petroleum feedstock by establishing a bio-based economy.

Principally, production and harvest of biomass plant is neither self-sustained nor environmentally friendly. It is a harvesting-out process of nutritious compound from field. Food waste and wastewaters, further, are unavoidably produced to pollute environment. So that, the total system design for recycle of all elements, not only carbon as neutral but also including nitrogen, potassium and phosphorus, is important for sustainable biomass production. Cascade utilization of biobased-products and recycle of biomaterials in a waste stream and wastewater, is another key technology for carbon sequestration and for the sustainable production-utilization system like metabolic network in human body.

We human beings are keeping our body function to be active by taking into the energy and chemicals as food. At the same time, we continuously use over half of total energy at our liver and pancreas, organs working in catabolism cleaning up our blood and recovering metabolites to maintain our body functions healthy. Treatment and utilization of waste materials may be compared with recycle of biomolecules via venous blood stream. In this context, our society has to further enrich the quality and quantity of 'venous industry' to treat waste and recover resources from them sustaining our society to be healthy.

Here, we present a total recycle system of food waste via chemical production with energy and facility savings and minimal emissions from waste materials. It should be further investigated to trait by improving the leading case study in 'Bio-economy system'. The challenge of the next decade will be to develop zero-emission bio-based environmentally friendly products from geographically distributed feedstock and worldwide generated food waste by simultaneous reduction of pollution indeed.

7. References

Addink, R. & Olie, K. (1995). Mechanisms of Formation and Destruction of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in Heterogeneous Systems. *Environmental Science and Technology,* 29, 1425-1435.

Bischoff, K. M.; Liu, S.; Hughes, S. R. & Rich, J. O. (2010). Fermentation of corn fiber and hydrolysate to lactic acid by the moderate thermophile *Bacillus coagulans*. *Biotechnology Letters,* 32, 823-828.

Boustead, I. (2002). *Eco-profiles of the European plastics industry: Polyethylene terephthalate.* Prepared for the European Centre for Plastics in the Environment, Brussels. www.apme.org

Camobreco, V.; Ham, R.; Barlaz, M.; Repa, E.; Felker, M.; Rousseau, C. & Rathle, J. (1999). Life-cycle inventory of a modern municipal solid waste landfill, *Waste Management and Research,* 17, 394–408.

Dijkhuizen, L. & Arfan, N. (1990). Methanol metabolism in thermotolerant methlotropic *Bacillus* species. *Federation of European Microbiological Societies, Microbiology reviews*, 87, 215-220.

Frederico, V. P.; Henry, P. F. & David, F. O. (1994). Kinetics and Modeling of Lactic Acid Production by *Lactobacillus plantarum*. *Applied and Environmental Microbiology*, 60, 2627-2636.

Harrison, K. W.; Dumas, R. D.; Barlaz, M. A. and Nishtala, S. R. (2000). A life-cycle inventory model of municipal solid waste combustion. *Journal of the Air and Waste Management* Association, 50, 993–1003.

Heriban, V.; Sturdik, E.; Zalibara, L. & Matus, P. (1993). Process and metabolic characteristics of *Bacillus coagulans* as a lactic acid producer. *Letters in Applied Microbiology*, 16, 243–246.

Hofvendahl, K. & Hagerdal, B.H. (2000). Factors affecting the fermentative lactic acid production from renewable resources. *Enzyme Microbial Technology*, 26, 87–107.

Kamm, B.; Gruber, P.R. & Kamm, M. (2005). *Biorefineries-Industrial Processes and Products*, Wiley-VCH, ISBN 3-527-31027-4, Germany

KBBE (2010). *The Knowledge Based Bio-Economy in Europe: Achievements and Challenges*. http://www.bio economy.net/reports/files/KBBE_2020_BE_presidency.pdf

Kitpreechavanich, V.; Maneeboon, T.; Kayano, Y. & Sakai, K. (2008). Comparative Characterization of L-Lactic Acid-Producing Thermotolerant *Rhizopus* Fungi. *Journal of Bioscience and Bioengineering*, 106, 541–546.

Madigan, M.T.; Martino, J.M.; Dunlap, P.V. & Clark, D.P. (Ed.). (2009). *Brock Biology of Microorganisms.* Pearson International, pp. 159, ISBN 0-321-5365-0, NewYork, USA

Miura, S.; Arimura, T.; Hosino, M.; Kojima, L.D. & Okabe, M. (2003). Optimization and scale-up of L-lactic acid fermentation by mutant strain Rhizopus sp.MK. *Journal of Bioscience and Bioengineering,* 96, 65-69.

Miyazaki, H. & Harano, K. (2001). Degradation test on biodegradable plastics polymers into composting. In: 2000 *Annual Research Report of Oita Industrial Research Institute*. Ono-Kousoku, Oita, Japan: Oita Industrial Research Institute, pp. 154–157

Mori, M.; Kuribayashi, M.; Nakamura, M.; Nishimura, Y.; Shira, Y. & Sakai, K. (2008). Material Balance in the Process of producing Poly-L-lactic acid from Municipal Food waste and Recycling the Byproduct as Organic Nitrogen Fertilizer for Rice Cultivation. *The Japan Society of Waste Management Exports*, 29, 400-408.

Nishida, H.; Fan, Y. ; Shirai, Y.; Tokiwa, Y. & Takeshi Endo T. (2004).Thermal degradation behaviour of poly(lactic acid) stereo complex. *Polymer Degradation and Stability*, 86, 197-208.

Oda, Y.; Park, B.; Moon, K. & Tonomura, K. (1997). Recycling of bakery wastes using an amylolytic lactic acid bacterium. *Bioresource Technology,* 60, 101–106.

Oh, H.; Wee, Y.J.; Yun, J.S.; Han, S.H.; Jung, S. & Ryu, H.W. (2005). Lactic acid production from agricultural resources as cheap raw materials. *Journal of Bioscience and Bioengineering*, 96, 1492–1498.

Ohara, H. & Sawa, S. (1994). Purification process of L-lactic acid for bio-degradable poly-L-lactate. In Biodegradable plastics and polymers, edited by Doi Y. & Fukuda K. Amsterdam: Elsevier

Ohara, H. & Yahata, M. (1996). L-Lactic acid production by *Bacillus* sp. in anaerobic and aerobic culture. *Journal of Fermentation and Bioengineering*, 81, 272–274.

Parfitt, J.; Barthel, M. & Macnaughton, S. (2010). Food waste within food supply chains: quantification and potential for change to 2050. *Philosophical Transactions of Royal Society*, 365, 3065-3081

Sakai, K.; Kudoh, E.; Wakayama, M. & Moriguchi, M. (2000a). Molecular analysis of microbial community in an activated sludge enriched by inorganic-nitrite medium. *Microbes Environment*, 15, 103–112.

Sakai, K.; Murata, Y.; Yamazumi, H.; Tau, Y.; Mori, M.; Moriguchi, M. & Shirai, Y. (2000b). Selective proliferation of lactic acid bacteria and accumulation of lactic acid during open fermentation of kitchen refuse with intermittent pH adjustment. *Food Science Technology*, 6, 140–145.

Sakai, K.; Kawano, H.; Iwami, A.; Nakamura, M. & Moriguchi, M. (2001). Isolation of a thermophilic poly-l-lactide degrading bacterium from compost and its enzymatic characterization. *Journal of Bioscience and Bioengineering*, 92, 298–300.

Sakai, K.; Mori, M.; Fujii, A.; Iwami, Y.; Chukeatirote, E. & Shirai, Y. (2004a). Fluorescent in situ hybridization analysis of open lactic acid fermentation of kitchen refuse using rRNA targeted oligonucleotide probes. *Journal of Bioscience and Bioengineering,* 98, 48–56.

Sakai, K.; Taniguchi, M.; Miura, S.; Ohara, H.; Matsumoto, T. & Shirai, Y. (2004b). Making plastics from garbage: a novel process for poly-L-lactate production from municipal food waste. *Journal of Industrial Ecology*, 7, 63–73.

Sakai, K.; Fujii, N. & Chukeatirote, E. (2006a). Racemization of L-lactic acid in pH-swing open fermentation of kitchen refuse by selective proliferation of *Lactobacillus plantarum*. *Journal of Bioscience and Bioengineering*, 102, 227–232.

Sakai, K. & Yamanami, T. (2006b). Thermotolerant *Bacillus licheniformis* TY7 Produces Optically active L-lactic acid from kitchen refuse under open condition. *Journal of Bioscience and Bioengineering*, 102, 132–134.

Sakai, K.; & Ezaki, Y. (2006c). Open L-lactic acid fermentation of food refuse using thermophilic *Bacillus coagulans* and fluorescence in situ hybridization analysis of microflora. *Journal of Bioscience and Bioengineering*, 101, 457–463.

Sakai, K.; Oue K.; Umeki, M.; Mori, M. & Mochizuki, S. (2006d). Species-specific FISH analysis of the cecal microflora of rat administered lactic acid bacterial cells. World Journal of Microbiology and Biotechnology, 22, 493–499.

Sakai, K.; Ezaki, Y.; Tongpim, S. & Kitpreechavanich,V. (2006e). High-Temperature L-Lactic Acid Fermentation of Food Waste under Open Condition and Its FISH Analysis of Its Micro Flora. *Kasetsart Journal (Natural Science*), 40, 35-39.

Sakai, K. (2007). Establishment of carbondioxide- free composting of municipal food waste with producing poly L-lactic acid plastic. *Soil microorganisms*, 61, 103-110.

Sakai, K. (2008). Production of Optically Active Lactic acid by using Non-LAB Microorganisms. White Biotechnology; *The front of Energy and Material Development*, Ohara, H. & Kimura Y. CMC publisher, pp. 98-108 ISSN, Tokyo, Japan (in Japanese)

Shirai, Y. & Sakai, K. (2006). *Utilization of Food refuse, Hand book of Food Engineering.* Asakura Shotan, pp. 460, ISBN 4-354-43091-K, Tokyo, Japan (in Japanese)

Umeki, M.; Oue K.; Mori M.; Mochizuki S. & Sakai K. (2005). Fluorescent *In Situ* Hybridisation Analysis of Cecal Microflora in rats simultaneously administrated Lactobacillus rhamnosus KY-3 and Cellobiose. *Food Science and Technology Research*, 11, 168-170.

Umeki, M.; Oue, K.; Mochizuki, S.; Shirai, Y. & Sakai, K. (2004). *Effect of Lactobacillus rhamnosus* KY-3 and Cellobiose as Synbiotics on Lipid Metabolism in Rats. *Journal of Nutritional Science and Vitaminology,* 50, 330-334.

United Nations Food and Agriculture Organization (FAO), (2011). Global food losses and food waste. International Congress, Rome. http://www.fao.org/fileadmin/user_upload/ags/publications/GFL_web.pdf

Vink, E. T. H.; Rabago, K. R.; Glassner, D. A. & Gruber, P. R.(2003). Applications of life cycle assessment to Nature Works polylactide (PLA) production. *Polymer Degradation and Stability*, 80, 403–419.

Zeikus, J. G. (1979). Thermophilic bacteria: ecology, physiology and technology. *Enzyme Microbial Technology*, vol. 1, IPS Business Press

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Biotechnology is the scientific field of studying and applying the most efficient methods and techniques to get useful end-products for the human society by using viable micro-organisms, cells, and tissues of plants or animals, or even certain functional components of their organisms, that are grown in fully controlled conditions to maximize their specific metabolism inside fully automatic bioreactors. It is very important to make the specific difference between biotechnology as a distinct science of getting valuable products from molecules, cells or tissues of viable organisms, and any other applications of bioprocesses that are based on using the whole living plants or animals in different fields of human activities such as bioremediation, environmental protection, organic agriculture, or industrial exploitation of natural resources. The volume Advances in Applied Biotechnology is a scientific book containing recent advances of selected research works that are ongoing in certain biotechnological applications. Fourteen chapters divided in four sections related to the newest biotechnological achievements in environmental protection, medicine and health care, biopharmaceutical producing, molecular genetics, and tissue engineering are presented.

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