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Valorisation of bakery waste for succinic acid production

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In this paper, bakery waste, including cakes and pastries from Starbucks Hong Kong, was evaluated for the potential of succinic acid (SA) production. Through simultaneous hydrolysis and fungal autolysis, both cake and pastry hydrolysates were found to be rich in glucose (35.6 and 54.2 g L^{-1}) and free amino nitrogen (685.5 and 758.5 g L^{-1}), whereas the protein hydrolysis yields were 23.2 and 22.5%, respectively. These cake and pastry hydrolysates, together with magnesium carbonate (10 g L^{-1}) were subsequently used as feedstock in *Actinobacillus succinogenes* fermentation, and the resultant SA concentrations were 24.8 and 31.7 g L^{-1}, respectively. A cation-exchange resin-based process (*via* vacuum distillation and crystallisation) was subsequently used to recover the SA crystals from fermentation broth, and a high SA crystal purity (96–97.7%) was obtained. Results of the present work successfully demonstrated the novel use of bakery waste as the generic feedstock for the sustainable production of SA as a platform chemical in food waste biorefinery.

Introduction

Food waste, one of the largest potions of municipal solid waste, has grown to be a global problem. Every year nearly 1.3 billion tons of food trash is dumped in landfills or is otherwise disposed of.[1] As one of the most densely populated cities worldwide, Hong Kong is greatly confronted with this problem. The booming economy and the metropolitan living style make this city the 'gourmet capital' of Asia. On the other hand, among developed countries in South East Asia, Hong Kong has the most imminent food waste management issue. The amount of food waste produced per person in Hong Kong is as high as 0.45 kg d^{-1}, which is much higher than that in Singapore (0.30 kg d^{-1}), South Korea (0.35 kg d^{-1}) and Taiwan (0.27 kg d^{-1}).[2] Accounting for 35.5% of total municipal solid waste disposal, the food waste in Hong Kong is currently incinerated or disposed of in landfills, which apparently increases the risk of soil and underground water pollution due to the leakage of organic compounds.[3] Besides, it is highly likely that existing landfills will be full before 2020 according to the local waste disposal plan.

To alleviate the pressure of the food waste crisis in Hong Kong, local researchers are seeking alternative waste valorisation strategies. Lin *et al.* pointed out that food waste could be a valuable resource for the production of chemicals, materials and fuels.[4] For example, the feasibility of fermentative poly-lactic acid production from bakery waste has been demonstrated recently, leading to the fabrication of a 100% biodegradable film.[5] This film can be used in the packaging of bakery products which closes the life cycle of bakery waste. Our research group has recently developed a novel food waste strategy for the fermentative production of a high-value product, namely succinic acid (SA) using bread waste.[6] In 2004 and 2010, SA was ranked as one of the top platform molecules by the U.S. Department of Energy.[7,8] Chemicals produced from SA include γ-butyrolactone, tetrahydrofuran, 1,4-butanediol, adipic acid, succinonitrile, succindiamide, 4,4-bionolle® and various pyrrolidones. The annual production of SA has reached 30 000–35 000 tonnes with a market value of US $225 million.[9] Double-digit growth rates are expected in the next five years.[10] Currently, a large number of companies such as BioAmber,[11] Reverdia[12] and Myriant[13] have already built demonstration plants for up-scaling the fermentative SA production from renewable feedstock. Lin *et al.* reported a wheat-based biorefining strategy for fermentative production and chemical transformations of SA.[4,14] Theoretically, two molecules of SA should be expected to be produced from one molecule of monosaccharide in fermentation.[15] In this study, we investigate the feasibility of utilising unconsumed bakery waste for fermentative SA production. The raw materials are donated by Starbucks Hong Kong under the 'Care for Our Planet Cookies' campaign which was implemented in

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April 2012.[16] This research has been featured by various international and local media such as Time Magazine,[17] the New York Daily News[18] and the South China Morning Post.[19] Therefore, this study provides an overview using bioprocessing strategies as an innovative solution for tackling the food waste issue in Hong Kong.

Materials and methods

Chemicals and micro-organisms

All chemicals used in this work were purchased from Acros Organics (Morris Plains, NJ, USA) and Sigma-Aldrich (St. Louis, MO, USA) except otherwise specified. Micro-organisms *Aspergillus awamori* (ATCC 14331) and *Actinobacillus succinogenes* (ATCC 55618) were obtained from the American Type Culture Collection (Rockville, MD, USA) for the production of amylolytic enzymes and for SA fermentation, respectively. An industrial strain of *Aspergillus oryzae* isolated from a soy sauce starter was kindly provided by the Amoy Food Ltd. (Hong Kong), which was utilised to produce proteolytic enzymes. Their storage and sporulation for inoculum preparation was conducted according to a technique reported previously.[20]

Raw materials

Unconsumed bakery waste was kindly provided by the Starbucks outlet at Shatin in Hong Kong. These in-kinds were donated for promoting the sustainability research as part of the 'Care for Our Planet' campaign. These wastes were divided into two groups: (1) pastry waste including apple Danish, croissants, mushroom and cheese pockets, Italian sausage sticks, sausage rolls and traditional cheese scones; and (2) cake pieces including red velvet cakes, chocolate marquise, blueberry cheese cakes and orange white chocolate dome cakes. Pastry waste and cake pieces were separately blended using a blender. Moisture and starch contents of these blended bakery wastes were analyzed as described by Koutinas *et al.*[21] Carbohydrates and lipids were determined as described by Pleissner and Eriksen.[22] Total nitrogen was determined through nitrogen analysis by a modified Kjeldahl digestion and Nesslerization method.[23,24]

Solid state fermentation (SSF)

Blended bakery waste was weighted prior to cultivation. The blended pastry or cake waste (10 g) was transferred into a Petri dish. Cryopreservation vials of *A. awamori* and *A. oryzae* were taken from −80 °C freezer and allowed to reach room temperature. Cryopreserved spores (1 mL) of *A. awamori* (2.85×10^7 spores mL^{-1}) or *A. oryzae* (6.31×10^6 spores mL^{-1}) were diluted by 10-fold and spread evenly onto food pieces. The prepared samples were incubated at 30 °C for 5 d.

Enzymatic hydrolysis

Simultaneous hydrolyses and fungal autolysis of pastry and cake waste was separately carried out in two 2.5 L bioreactors (BioFlo/CelliGen 115, New Brunswick Scientific, Edison, NJ, USA). The bioreactors were equipped with automatic temperature control water jacket and stirrers. Various amounts of food waste were blended with 1 L of water for 15 min. The resulting mixture was transferred into the bioreactors set at 55 °C. Fungal mashes from SSF were added into the vessel. The reaction mixture was stirred at 300 rpm, whereas the pH was not controlled during hydrolysis. Hydrolysis samples were taken every 1 h for 24 h. The cell slurry was centrifuged for 1 h at 10 000 rpm and 4 °C. The supernatant was subsequently filtered by vacuum filtration using Whatman No. 1 filter paper. The resultant solution was kept at −20 °C in a freezer and was subsequently used as the sole fermentation medium for SA fermentation. All experiments were carried out in duplicate.

Bacterial fermentation

Two 2.5 L fermentors (Biostat, Sartorius stedim, Germany) were used for SA production. The initial glucose concentrations of pastry and cake hydrolysates were 44.0 and 23.1 g L^{-1}, respectively, and the free amino nitrogen (FAN) concentrations were 715 and 388 mg L^{-1}, respectively. The hydrolysate was filtered by 0.2 μm PTFE membrane filter (Sartorius, Germany). Seed culture was inoculated (5% v/v) into the fermentation medium. The sterilized magnesium carbonate ($MgCO_3$) (10 g L^{-1}) was added to the filtered fermentation medium as a neutral pH buffer. Fermentation was conducted under 37 °C with external CO_2 sparging into the broth at a rate of 0.5 vvm and reactor agitating at 300 rpm. The pH of the fermentation broth was automatically controlled within 6.6–6.8 with the addition of NaOH (10 M) and H_2SO_4 (0.5 M). Fermentation samples were taken every 3 h to measure optical density and metabolites concentration. Fermentations were considered finished either when glucose was completely depleted or when no change in total sugar concentration was detected for a period of 5 h.

Recovery of succinic acid crystals

The recovery of SA crystals from the fermentation broth was carried out as previously described with minor modification.[25] The fermentation broth (100 mL) was centrifuged at 7500 rpm and 4 °C for 1 h, and the supernatant was filtrated through Whatman No. 1 paper. The filtrate was then mixed with active carbon (12.5%, w/v) for 2 h to remove the organic impurities. The mixture was centrifuged for 1 h at 4 °C and the resultant supernatant was filtered through Whatman No. 1 paper. The colorless solution obtained was further treated using the cation-exchange resin Amberlite IR 120H as described in ref. 25. The solution was vacuum distilled at 74 °C and −78 kPa until a significant amount of condensate was collected. The crystallization was subsequently carried out at 4 °C for 24 h. The final slurry was filtered through Whatman No. 1 paper and the SA crystals were dried at 55 °C for 12 h.

Determination of cell density, sugar concentration, free amino nitrogen (FAN) and fermentation metabolites

The detection of bacterial growth was carried out as previously described with minor modification.[26] Bacterial growth within the fermentation samples was determined by optical density (OD) measurements at a wavelength of 660 nm using the spectrophotometer (UV-1800, Shimadzu, Japan). Glucose and metabolite concentrations were quantified using the high performance liquid chromatography (HPLC) system (Waters, UK), which was equipped with a BIO-RAD column (HPX-87H), a refractive index (RI) detector (Waters, UK) and a photodiode array (PDA) analyser (Waters, UK). The mobile phase was H_2SO_4 (5 mM) with a flow rate of 0.6 mL min^{-1}. The column and RI detector were equilibrated to 65 and 35 °C, respectively. Injection volume of all samples and standards was set to 20 μL. FAN concentration of the hydrolysis samples was analyzed using the ninhydrin colorimetric method promulgated in the 1987 European Brewery Convention.[26]

Results and discussion

Bakery waste composition

The composition of bakery waste is presented in Table 1. Results showed that both pastry and cake waste were rich in carbon and nitrogen sources, and hence these could be good source of nutrients. The starch content in pastry (44.6 g per 100 g) was higher than that in cake (12.6 g per 100 g), and pastry could be hydrolysed into generic medium with higher concentration of reducing sugars.

Enzymatic hydrolysis of food waste into fermentation feedstock

SSF solids of *A. awamori* and *A. oryzae* on bakery waste were utilised for the production of enzyme complexes (*i.e.* amylolytic enzymes and protease) that convert the raw material into a generic fermentation medium. Due to the absence of free water and low protein breakdown, SFF is an ideal method to allow the high enzyme concentration.[27] In enzymatic hydrolysis, the nutrients in pastry and cake in the form of macromolecules can be broken down into utilisable forms such as sugars and amino acids, which are required for the subsequent SA fermentation by *A. succinogenes*.[6,26]

Table 1 Composition of blended bakery waste (per 100 g)

	Bread[a]	Cake	Pastry
Moisture	22.3 g	45.0 g	34.5 g
Starch (dry basis)	59.8 g	12.6 g	44.6 g
Carbohydrate	46.8 g	62.0 g	33.5 g
Lipids	0.9 g	19.0 g	35.2 g
Sucrose	3.0 g	22.7 g	4.5 g
Fructose	—	11.9 g	2.3 g
Protein (TN × 5.7) (dry basis)	8.9 g	17.0 g	7.1 g
Total phosphorus (dry basis)	Trace	1.5 g	1.7 g
Ash (dry basis)	—	1.6 g	2.5 g

[a] Ref. 6.

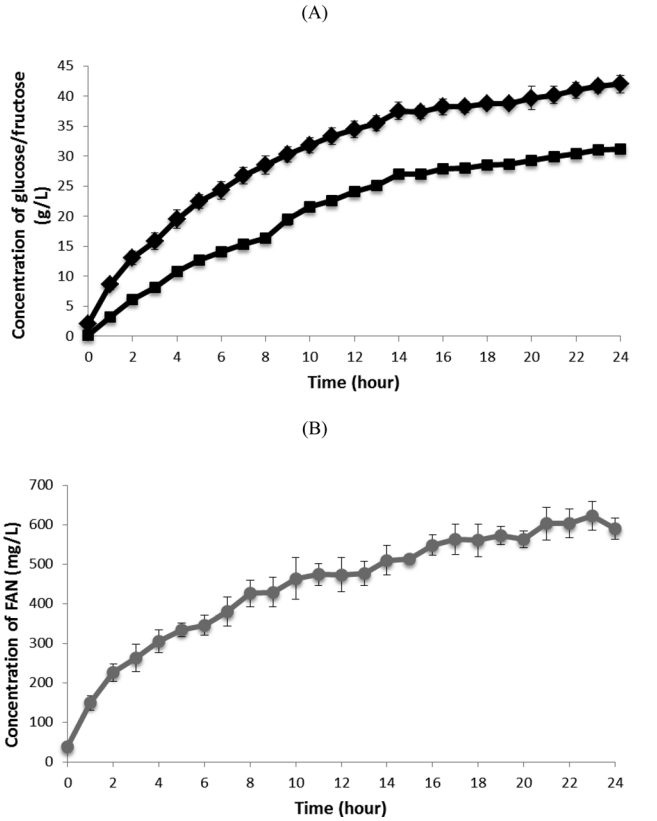

Fig. 1 (A) Sugar and (B) FAN production from simultaneous hydrolytic/autolytic reactions using 30% cake (w/v, dry basis). Hydrolysis was carried out at 55 °C. Average values and error bars of the duplicate experiments of each condition are shown. (◆) Glucose; (■) fructose; (●) FAN.

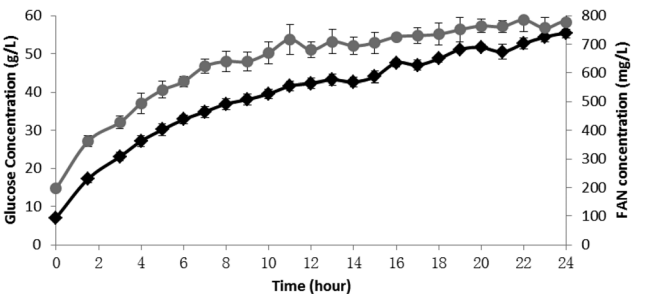

Fig. 2 Glucose and FAN production from simultaneous hydrolytic/autolytic reactions using 30% pastry (w/v, dry basis). Hydrolysis was carried out at 55 °C. Average values and error bars of the duplicate experiments of each condition are shown. (◆) Glucose; (●) FAN.

The yields of sugars and FAN from the hydrolysis of bakery waste were shown in Fig. 1 and 2. With an initial 30% (w/v) substrate concentration, both sugars and FAN almost reached their saturation concentrations in 24 h. Such a hydrolysis duration was consistent with our previous findings using bread waste[6] and wheat flour milling by-products.[26] As the pastry contains more starch, the yields of glucose and FAN in the

Table 2 Sugar and FAN concentrations achieved from enzymatic hydrolysis using different bakery waste (30%, w/v)

	Bread[a]	Cake	Pastry
Glucose (g L^{-1})	104.8	35.6	54.2
Fructose (g L^{-1})	—	23.1	Trace
Total nitrogen (g L^{-1})	1.61	1.22	2.28
FAN (mg L^{-1})	492.6	282.3	517.4
FAN/TN ratio (%)	30.5	23.2	22.7

[a] Ref. 6.

pastry hydrolysate were higher than those in cake hydrolysate. According to Table 2, the glucose (54.2 g L^{-1}) and FAN concentrations (758.5 mg L^{-1}) were achieved at 30% (w/v) pastry waste after enzymatic hydrolysis. On the other hand, the sucrose, a disaccharide present in cake, was hydrolysed to form 1 mol of glucose and 1 mol of fructose. The glucose (35.6 g L^{-1}), fructose (23.1 g L^{-1}) and FAN concentrations (685.5 mg L^{-1}) were achieved at 30% (w/v) cake waste. The protein hydrolysis yields (FAN/TN ratio) were 23.2 and 22.7% for cake and pastry hydrolysates, respectively. These values were similar to that previously reported by Wang,[28] in which 20.5% of nitrogen (TN) from the wheat four was converted and released into the liquid phase in the form of FAN. The results clearly demonstrated the potential of utilising bakery hydrolysate as the generic feedstock for the subsequent SA fermentation.

Batch fermentation of succinic acid

Batch fermentation on enzymatic hydrolysates was carried out to investigate cell growth, glucose consumption and SA production. According to Table 3 and Fig. 3, cake hydrolysate with an initial sugar content of 23.1 g L^{-1} glucose and 18.5 g L^{-1} fructose, and pastry hydrolysate with an initial sugar content of 44.0 g L^{-1} glucose were both utilised as the fermentation feedstock. As shown in Fig. 3(A), the amounts of glucose and

Table 3 Succinic acid fermentation of *A. succinogenes* on various hydrolysates produced from bakery waste

	Bread[a]	Cake	Pastry
Initial content of fermentation media			
Glucose (g L^{-1})	39.6	23.1	44.0
Fructose (g L^{-1})	—	18.5	Trace
Total sugar (g L^{-1})	39.6	41.6	44.0
FAN (mg L^{-1})	200	388	715
Yeast extract equivalent (g L^{-1})	4.0	7.76	14.30
Fermentation outcome			
SA concentration (g L^{-1})	47.3	24.8	31.7
Yield (g SA g^{-1} total sugar)	1.16	0.80	0.67
SA productivity (g SA L^{-1} h^{-1})	1.12	0.79	0.87
Overall conversion yield (g SA g^{-1} substrate)	0.55	0.28	0.35
Glucose consumption (g L^{-1})	39.1	17.9	43.8
Glucose utilisation (g g^{-1})	0.99	0.76	1.0
Fructose consumption (g L^{-1})	—	14.8	—
Fructose utilization (g g^{-1})	—	0.79	—
Total sugar consumption (g L^{-1})	39.1	32.6	43.8
Total sugar utilization (g g^{-1})	0.99	0.77	1.0

[a] Ref. 6.

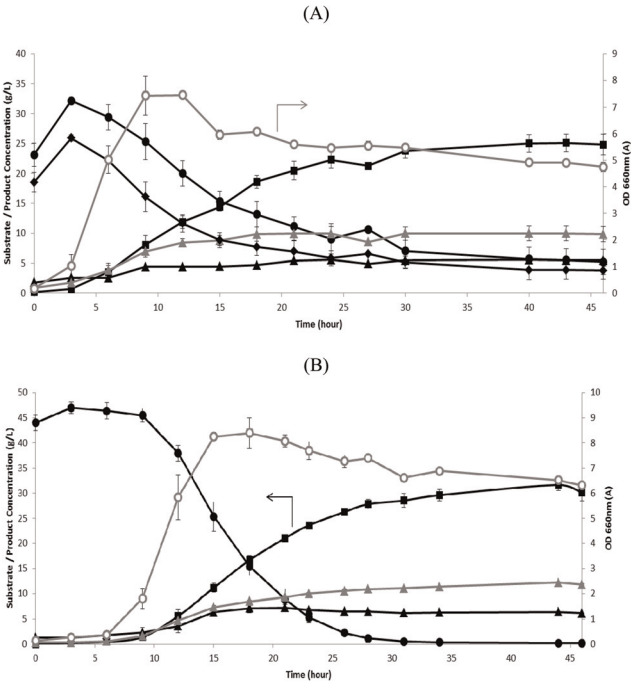

Fig. 3 SA fermentation using (A) cake and (B) pastry hydrolysates. The average concentrations and error bars of the duplicated experiments are shown. (●) Glucose; (◆) fructose; (■) SA; (▲) acetate; (▲) formate; (○) OD_{660}.

fructose started to decline after 5 h and they were consumed simultaneously in the fermentative production of SA using cake hydrolysate. Without any additional supplement of carbon or nitrogen sources, a significant increase in OD_{660} was observed (from 0 to 5.02) within 6 h, indicating the cake hydrolysate had sufficient nutrients to support the growth of *A. succinogenes*. The growth reached a maximum OD_{660} of 7.45 at 12 h, and then started to decline gradually. During the stationary phase, the average OD_{660} was 5.5. The production of SA started from the early exponential phase and continued until sugar was depleted after 30 h. The fermentation was completed after 40 h when the total sugar concentration remained unchanged. The remaining glucose was 5.2 g L^{-1} whilst fructose was 3.7 g L^{-1}. A final SA concentration of 24.8 g L^{-1} was obtained at the end point, which corresponded to a yield of 0.8 g SA g^{-1} total sugar and a productivity of 0.79 g L^{-1} h^{-1}. The overall conversion of waste cake into SA was 0.28 g g^{-1} cake.

Compared with cake hydrolysate, pastry hydrolysate had a higher concentration of initial glucose (44.0 g L^{-1}). As shown in Fig. 3(B), during the exponential phase (6–12 h), the OD_{660} showed a significant increase from 0.3 to 5.8. Glucose concentration also started to decline after 6 h. At 18 h, cell growth attained a maximum OD_{660} of 8.4. The SA concentration continuously increased until sugar was depleted after 44 h. At the end of fermentation, the SA concentration reached 31.7 g L^{-1}, which corresponded to a yield of 0.67 g SA g^{-1} glucose and a productivity of 0.87 g L^{-1} h^{-1}. SA production achieved from various food wastes is compared and summarised in Table 4.

Table 4 Comparison of succinic acid yields achieved using different food waste substrates

Substrate	SA yield (g SA g^{-1} TS)	Overall SA yield (g SA g^{-1} substrate)	References
Wheat	0.40	0.40	20
Wheat flour milling by-product	1.02	0.087	26
Potatoes	—	—	29
Corncob	0.58	—	30
Rapeseed meal[a]	0.115	—	31
Rapeseed meal[b]	—	—	32
Orange peel	0.58	Negligible	33
Bread	1.16	0.55	6
Cake	0.80	0.28	This work
Pastry	0.67	0.35	This work

[a] Rapeseed meal is treated by diluted sulphuric acid hydrolysis and subsequent enzymatic hydrolysis of pectinase, celluclast and viscozyme. [b] Rapeseed meal is treated by enzymatic hydrolysis using *A. oryzae*.

It is clear that when using cake and pastry wastes as the feedstocks, the SA yields obtained are comparable or higher than other food waste-derived media.

Succinic acid recovery using vacuum distillation and crystallisation

A cation-exchange resin-based process *via* vacuum distillation and crystallisation was employed to recover the SA crystals from bakery hydrolysate fermentation broth produced by *A. succinogenes*. As illustrated in Table 5, high purities of SA crystals were obtained for both pastry (97.7%) and cake hydrolysates (96.0%), which were even higher than in semi-defined medium (92.3%). Also, these results were comparable to our previous study using wheat-derived fermentation broth (SA crystal purity: 99%).[25] The SA crystals were shown in Fig. 4. According to Lin *et al.*,[4] the future advanced food waste valorization practices aiming to achieve sustainable development and a zero-waste economy should focus on innovative low environmental impact legislation-compliant technologies able to convert waste from the biorefinery process into value-added products. These include anaerobic digestion (hailed as the future of food waste management), low environmental impact chemical technologies, integrated bio-chemical processing approaches, extractive processes for the recovery of valuable compounds, and so on.

Table 5 Summary of succinic acid crystals recovered using various media. An initial volume of 100 mL was used for all fermentation broths

	Semi-defined medium	Cake	Pastry
Initial SA concentration (g L^{-1})	15.0	24.8	31.6
SA recovery (%)	39.4	16.2	20.9
Purity (%)	92.3	96.0	97.7

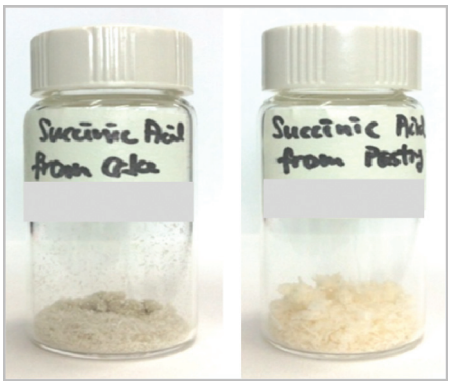

Fig. 4 SA crystals recovered from (left) cake and (right) pastry hydrolysates.

Conclusions

Results of the present study demonstrated that bakery waste can be successfully used as the nutrient-complete hydrolysates for the fermentative production of SA. The overall SA produced from pastry and cake wastes was 0.35 and 0.28 g g^{-1} substrate, respectively, and high recovery yields of SA crystals (96–98%) were obtained. This is the first report to present a complete bioconversion process utilising bakery waste for SA crystal production. A novel food waste biorefinery concept named 'Starbucks Biorefinery' is therefore developed, which has received over 66 700 hits in Google search as of September 25, 2012. This research suggests that bakery waste has an enormous potential as a renewable resource for producing chemicals and materials. Continued efforts should be devoted to develop pilot-scale plants using these 'Starbucks Biorefinery' strategies for solving global food waste issue.

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