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Enhanced production of lactic acid with reducing excess sludge by lactate fermentation

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ABSTRACT

The development of a facile technology for utilizing effectively and/or reducing excess sludge is one of the urgent problems since a large quantity of sewage sludge is formed by activated sludge processes. Excess sludge containing 50 mM sucrose was fermented at 50 °C using endogenous bacteria in excess sludge, resulting in a high lactic acid production (8.45 g/L) and in an increased sludge reduction (38.2%). Conversion rate to lactic acid was up to 106.0% by standard fermentation at 50 °C compared to 43.8% at 30 °C and this phenomenon that conversion rate was higher was observed only at 50 °C as the fermentation at less or more than 50 °C had lower conversion rate than that at 50 °C. Lactic acid bacteria increased at 50 °C during 1-d fermentation whereas the number of total viable bacteria only increased slightly, indicating that lactic acid bacteria in sludge at 50 °C were preferentially able to utilize the sucrose for producing lactic acid. Finally, pH-vibration fermentation at 50 °C enabled to completely consume residual sucrose in the normal fermentation, resulting in the maximum production of lactic acid. Lactate fermentation by a purely cultured lactic acid bacterium TS1 with autoclaved excess sludge containing 50 mM sucrose had more than 100% of conversion rate to lactic acid, indicating that a part of sludge was converted into lactic acid during the fermentation. Our technique is useful as a facile engineering for reducing excess sludge concomitantly with producing lactic acid by lactate fermentation.

1. Introduction

Municipal sewage and industrial wastewater containing organic matters are treated by an aerobic biological treatment as an activated sludge process [1]; however, the process has formed a large quantity of sewage sludge such as primary sludge and waste activated sludge [2,3]. The activated sludge process is a continuous system; bacteria in the sludge are mixed with wastewater, and then the mixtures are aerated to facilitate the degradation of organic matters into wastewater and separated in a gravity clarifier (the clarified water disembogues into the environment). A portion of the concentrated sludge is recycled and mixed with additional wastewater. The residual sludge (called excess sludge) is a complex aggregation of microorganisms [4], and is discharged. The quantity of the generated excess sludge has been increasing each year with the development of sewer systems (http://www.jswa.jp/05_arekore/06_use/riyou/data.html); therefore, the treatment and disposal of the excess sludge is of utmost importance for developing an environmental burden-reducing technology in small countries such as Japan where the availability of landfill sites is declining [5], as well as a high cost of sludge disposal [6]. Also, it is extremely difficult to dehydrate excess sludge using a chemical conditioning since there is a large fraction of bound water in the excess sludge, and then to dump, incinerate, or bury excess sludge due to the shortage of landfills and terrible odor emission [7,8]; hence, the disposal and treatment of excess sludge has been considered to be a serious social problem. Therefore, developing an effective disposal technique to reduce excess sludge as soon as possible is actually essential for overcoming such a serious problem on excess sludge. To date, many studies to prevent the generation of excess sludge or to reduce excess sludge formed have been reported; for example, ultrasonic treatment [9,10], activated sludge ozonation [11], chemical treatment using tetrachlorosalicylanilide (TCS) [12], thermal processing [13], mechanical disintegration [14], and alkaline thermal sludge hydrolysis [15] as chemical approaches, and biological solubilization of organic sludge by thermophilic aerobic bacteria [16] and by Oligochaeta [17]. On the other hand, hydrogen [18], poly-β-hydroxybutyrate [19,20], polyhydroxylalkanoate production [21,22], methane fermentation [23], and eco-cement [24] are useful for recycling excess sludge.

Lactic acid has been in use as a natural preservative in many food products since a long time ago [25]. Currently, lactic acid is

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Table 1
Chemical composition of waste activated sludge in the Hiagari sewage disposal plant.

Parameter	Waste activated sludge
TS (%)	3.3–4.0
VS (%)	2.6–3.1
SS (g/L)	30–41
VSS (g/L)	26–33
COD_{Cr} (g/L)	44–51
Protein (% of TS)	40–45
Carbohydrate (% of TS)	12–14
Lipid (% of TS)	11–13

TS: total solid; VS: volatile solid; SS: suspended solid; VSS: volatile suspended solid; COD_{Cr}: chemical oxygen demand.

used in a wide variety of the specialized industrial applications; lactic acid has the potential of becoming a very large volume, commodity-chemical intermediate produced from renewable carbohydrates for the use as feedstocks for biodegradable polymers, oxygenated chemicals, plant growth regulators, environmentally friendly 'green' solvents, and specialty chemical intermediates [26]. Therefore, the demand for lactic acid is expected to increase as rated by different surveys due to its use in biodegradable plastics and other large-scale industrial products. Also, lactic acid has been used for the healthy lifestyles of human; hence, to date, many foods containing lactic acid such as yogurt [27], yakult [28], and bread [29] have been developed. Currently, many studies on probiotics to improve further human health have been reported [30] as the inhibition of pathogen attachment [31] and cholesterol reduction by lactic acid bacteria [32]; hence, lactic acid bacteria will be one of the beneficial bacteria in many industrial fields. Lactic acid is produced from various biomass such as garbage [33,34], potato starch [35], and lignocellulosic biomass [36,37], as well as the molecule is manufactured either by chemical synthesis or by microbial fermentation; however, there are no reports on the production of lactic acid from excess sludge to the present date. In this paper, our goal was to examine the reduction of excess sludge through lactic acid fermentation using endogenous bacteria in excess sludge.

2. Materials and methods

2.1. Materials

Excess sludge used in this study was freshly obtained from the Hiagari sewage disposal plant located in Kitakyushu, Japan, which formed 7530 m^3 of excess sludge daily. The chemical composition of waste activated sludge is shown in Table 1. Standard methods [38] were used for the determination of total solid (TS), volatile solid (VS), suspended solid (SS), volatile suspended solid (VSS), and chemical oxygen demand (CODCr). Protein, carbohydrate, and lipid concentrations were determined by the Lowry's method [39] using bovine serum albumin as a standard chemical, by the phenol-sulfuric acid method [40] using glucose as a standard chemical, and by the Bligh–Dyer method [41], respectively. All chemicals were of the highest purity commercially available.

2.2. Lactate fermentation

The best mix proportion, excess sludge (wet weight: 160 g) and distilled water (80 mL) were mixed, and then sucrose (final concentration 0–50 mM), glucose, fructose, lactose, or maltose (final concentration 50 mM) was added into the mixture. The final mixture was incubated at 30–60 °C without shaking to avoid the depression of fermentation efficiency by aeration. Also, pH in excess sludge was measured with an Ion meter IM-40S (Toa Electronics, Tokyo, Japan).

2.3. Measurements of sludge weight, sugar, and organic acid

Sludge weight was measured as follows. Excess sludge (wet weight: 25 g) before or after fermentation was centrifuged at 18,000 × *g* for 10 min. The pellets were harvested on a vaporizing bowl and dried at 105 °C for 2 d with a Drying oven DO-300 (Asone, Osaka, Japan) to measure the dry weight. Sludge-reduction rate was calculated by the following formula: sludge-reduction rate (%) = (the dry weight before fermentation − the dry weight after fermentation (g))/the dry weight before fermentation (g) × 100.

Sugar concentrations were measured using the phenol-sulfuric acid method, as previously described [40]. The absorbance at 490 nm was measured with a UV/vis Spectrophotometer V-530 (Jasco, Tokyo, Japan).

Organic acids (lactic acid, succinic acid, formic acid, acetic acid, propionic acid, isobutyric acid, and butyric acid) were analyzed by high-performance liquid chromatography (HPLC). HPLC analyses were performed on a Shim-pack SCR-102H (8 mm × 300 mm; Shimadzu, Kyoto, Japan) with p-toluenesulfonate (5 mM) as a mobile phase, with a flow rate of 0.8 mL/min. All organic acids were detected with a Shimadzu CDD-6A electric conductivity detector.

2.4. Calculation of conversion rate from sugar to lactic acid

Conversion rate from sugars (glucose, galactose, fructose, xylose, lactose, maltose, and sucrose) to lactic acid was calculated as follows. Lactic acid fermentation generally produces 2 mol of lactic acid from 1 mol of glucose (i.e. 2 mol of lactic acid from monosaccharide and 4 mol of lactic acid from disaccharide).

Conversion rate to lactic acid = [lactic acid concentration (g/L)/90 (g/mol)]/[consumed sugar concentration (g/L)/molecular weight of sugar used (e.g. 180.16 g/mol for glucose and 342.30 g/mol for sucrose) × 1 (monosaccharide) or 2 (disaccharide) × 2].

2.5. Culturable viable cell counting

Culturable viable bacteria and lactic acid bacteria were measured as follows. Serial dilutions from the fermented solution (1 mL) were spread on standard medium agar plates (per liter: 5 g Bacto peptone, 2.5 g Bacto yeast extract, 1 g glucose, and 15 g agar in distilled water) for counting culturable viable bacteria, and were spread on MRS agar plate (Oxoid, Hampshire, England) containing 0.5% calcium carbonate for counting culturable lac-

Table 2
Lactate fermentation at 50 °C by excess sludge with different sugar (initial concentration 50 mM). Standard deviations are shown from three independent experiments.

	Sugar						
	Glucose	Galactose	Fructose	Xylose	Lactose	Maltose	Sucrose
Residual sugar concentration (mM)[a]	4.8 ± 0.1	9.3 ± 0.4	4.2 ± 0.1	4.1 ± 0.2	26.9 ± 0.5	29.4 ± 0.3	27 ± 0.2
Lactic acid concentration (g/L)[a]	5.9 ± 0.1	4.8 ± 0.2	4.3 ± 0.7	1.5 ± 0.3	7.14 ± 0.2	8.5 ± 0.4	8.9 ± 0.3
Sludge-reduction rate (%)	39.3	37.6	34.0	37.7	38.3	38.7	38.2
Conversion rate to lactic acid (%)	72.8	65.7	52.4	21.6	86.0	114.9	108.2
Relative	1	0.9	0.7	0.3	1.2	1.6	1.5

[a] After 5 d.

Table 3
Lactate fermentation at 50 °C with excess sludge containing different sucrose concentration. Standard deviations are shown from three independent experiments.

	Initial sucrose concentration (mM)			
	50	40	30	20
Residual sucrose concentration (mM)[a]	28 ± 3	18 ± 4	10 ± 1	6 ± 1
Lactic acid concentration (g/L)[a]	8.5 ± 0.8	8.2 ± 0.3	7.7 ± 0.5	5.1± 0.4
Initial pH	6.87	6.88	6.87	6.86
Final pH[a]	3.22	3.25	3.26	3.32
Conversion rate to lactic acid (%)	106.0	105.5	104.6	99.1
Sludge-reduction rate (%)	38.2	34.0	33.7	32.2

[a] After 5 d.

tic acid-producing bacteria. These plates were incubated at 37 °C for 2 d, and then colonies grown were counted. For organic acid-producing bacteria, colonies forming clear zone were counted. The decrease of pH in the media through the production of organic acid including lactic acid is responsible for the formation of clear zones on the plates. Also, to verify whether the clones that formed a clear zone are lactic acid bacteria, the candidate clones were tested with API 50CHL (Biomérieux, Tokyo, Japan).

2.6. pH-vibration fermentation

Excess sludge (wet weight: 160 g) and distilled water (80 mL) were mixed, and then sucrose (50 mM) was added into the mixture. The final mixture was incubated at 50 °C without shaking. Then, pH in excess sludge was adjusted to 6.8 for every 24 h with 25% aqueous ammonia since bacteria can utilize it as a nitrogen source.

2.7. Sludge reduction at 30 °C or 50 °C with or without adding lactic acid initially

Excess sludge (wet weight: 160 g) and distilled water (80 mL) were mixed, and prior to addition of sucrose (final concentration 50 mM), pH in the mixture was adjusted with or without adding lactic acid; thereby, the mixtures were adjusted at pH 3.2 or pH 6.8. The final mixture was incubated at 30 °C or 50 °C without shaking.

2.8. Amylase and cellulase activity in excess sludge

Excess sludge (wet weight: 160 g) and distilled water (80 mL) was mixed, and the mixture was incubated at 50 °C for 1 d. Then amylase and cellulase activity in the supernatant of excess sludge were measured as described previously [42]. All the enzyme assays were set up in duplicate.

2.9. Lactate fermentation by isolated lactic acid bacterium

A lactic acid bacterium (named *Lacto. acidophilus* strain TS1, one of the 12 clones isolated preferentially from the fermented samples for 1 d at 50 °C) was grown with MRS medium at 50 °C for 1 d, and then the cells were harvested from 2 mL of the cell culture by the centrifugation at 8000 × *g* for 1 min, washed twice by using 0.85% NaCl, and resuspended in the same buffer (1 mL). Excess sludge (wet weight: 160 g) and distilled water (80 mL) were mixed and autoclaved for 20 min at 121 °C (Tomy Seiko, Tokyo, Japan) to sterilize the mixture. Then, sucrose (final concentration 50 mM) and the cells suspension (1 mL) were added into the mixture. The final mixture was incubated for 4 d at 50 °C without shaking.

2.10. Screening of sludge-lysing bacteria

Excess sludge (160 g) and distilled water (80 mL) was mixed, and the mixture was incubated for 1 week. Then, the double volume of fresh excess sludge was mixed to single volume of the incubated excess sludge to increase the number of sludge-lysing bacteria (enrichment culture). The incubation was repeated every week (up to 8 weeks). After incubation, the serial dilutions from the cultures were spread on 2.5% (w/v)-autoclaved sludge agar plate containing 0.2% glucose. The plates were incubated at 50 °C for 1 d. The colonies that formed a sludge-lysing halo were isolated and used for the next experiment.

2.11. Sludge reduction by sludge-lysing bacteria

Sludge-lysing bacteria were grown with LB medium (per liter: 10 g Bacto Tryptone, 5 g Bacto yeast extract, and 5 g sodium chloride) at 50 °C for 1 d, and then the cells were harvested from 2 mL of the cell culture by the centrifugation at 8000 × *g* for 1 min, washed twice by using 0.85% NaCl, and resuspended in the same buffer (1 mL). Autoclaved excess sludge (160 g) and sterilized water (80 mL) was mixed, and the cell suspension (1 mL) was added to the mixture. The final mixture was incubated for 5 d.

3. Results

3.1. Lactate fermentation from excess sludge containing various sugars

We performed lactate fermentation at 50 °C using excess sludge containing various sugars (monosaccharide such as glucose, galactose, fructose, and xylose and disaccharide such as lactose, maltose, and sucrose) to examine the effect of reducing excess sludge and of synthesizing a beneficial product such as lactic acid, which is a material for biodegradable plastics. As shown in Table 2, lactic acid was produced by endogenous lactic acid bacteria in excess sludge and the lactate production from disaccharide had higher conversion

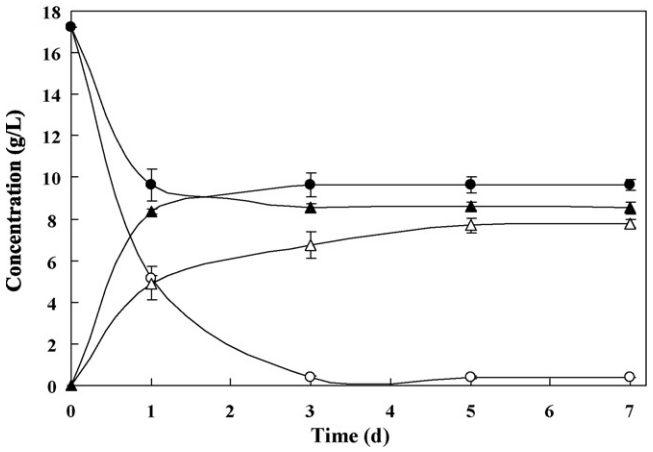

Fig. 1. Lactic acid fermentation at 30 °C (open symbols) and at 50 °C (solid symbols) by excess sludge containing 50 mM sucrose. Sucrose (circle) and lactic acid concentration (triangle) in the supernatant of excess sludge was analyzed during the fermentation. Standard deviations are shown from three independent experiments.

Table 4
Lactate fermentation by excess sludge with sucrose (50 mM) at different temperature. Standard deviations are shown from three independent experiments.

	Fermentation temperature (°C)			
	30	40	50	60
Residual sucrose concentration (mM)[a]	1.5 ± 0.8	1.5 ± 1.2	28 ± 3	39 ± 1
Lactic acid concentration (g/L)[a]	7.7 ± 0.2	8.4 ± 0.2	8.5 ± 0.8	1.6 ± 0.3
Sludge-reduction rate (%)	35.5	37.1	38.2	39.1
Conversion rate to lactic acid (%)	43.8	47.8	106.0	41.4
Relative	1	1.1	2.4	1

[a] After 5 d.

Table 5
Lactate fermentation by excess sludge with sucrose (50 mM) at different temperature. Standard deviations are shown from three independent experiments.

	Fermentation temperature (°C)						
	44	46	48	50	53	56	60
Residual sucrose concentration (mM)[a]	1.8 ± 0.8	11 ± 2	15 ± 2	28 ± 3	29 ± 4	36 ± 2	39 ± 1
Lactic acid concentration (g/L)[a]	8.3 ± 0.6	8.2 ± 0.4	8.1 ± 0.4	8.5± 0.8	7.1 ± 0.8	2.1 ± 0.6	1.6 ± 0.3
Initial pH	6.87	6.88	6.87	6.86	6.87	6.86	6.87
Final pH[a]	3.22	3.25	3.26	3.32	ND[b]	ND	ND
Conversion rate to lactic acid (%)	47.4	57.4	64.7	106.0	96.2	41.7	41.4
Sludge-reduction rate (%)	36.0	36.9	37.4	38.2	38.1	38.7	39.3

[a] After 5 d.
[b] Not determined.

rates to lactic acid than that from monosaccharide. In particular, the conversion rate from sucrose was more than 100% as well as from maltose; hence, sucrose which is at least 12 times cheaper than maltose (890 yen/kg for sucrose vs. 11,400 yen/kg for maltose; Nacalai Tesque, Kyoto, Japan) was used in the subsequent experiments.

3.2. Lactate fermentation from excess sludge containing sucrose

We examined lactate fermentation from excess sludge with the different concentration of sucrose and the lactic acid production enhanced according to an increase in the initial sucrose concentration (Table 3). Also, pH value in excess sludge decreased down to pH 3.2 by the production of lactic acid and conversion rate from sucrose to lactic acid showed more than 100% in excess sludge with more than 30 mM of sucrose concentration (Table 3), indicating that a part of excess sludge was converted into lactic acid by the fermentation at 50 °C. As corroborating evidence, the conversion rate was related to sludge-reducing rate, as shown in Table 3. In the fermentation at 50 °C, sucrose in excess sludge was not consumed after 1 d whereas complete consumption of sucrose was observed after 1 d in the fermentation at 30 °C (Fig. 1), although the change of pH was the same in the fermentation at both 30 °C and 50 °C. Lactic acid production in the fermentation at 50 °C was higher than that at 30 °C (Fig. 1 and Table 4). Conversion rate from sucrose to lactic acid was 106% at 50 °C compared to 43.8% at 30 °C, as shown in Table 4; conversion rate at 50 °C was 2.4 times higher than that at 30 °C. Also, sludge-reduction rate at 50 °C was slightly higher than that at 30 °C.

3.3. Effect of temperature in lactic acid fermentation

We examined the effect of incubation temperature in lactate fermentation to reveal which temperature is the best fermentation condition. Concentration of lactic acid, which was produced from excess sludge including sucrose, was closely in the fermentation at 30, 40, and 50 °C, except low lactate concentration at 60 °C (Table 4). Sucrose in excess sludge was less consumed at 50 and 60 °C compared to at 30 and 40 °C, showing mostly the consumption of sucrose. The phenomenon, in which conversion rate from sucrose to lactic acid was more than 100%, was specific to the fermentation at 50 °C compared with those at other temperatures (30, 40, 44, 46, 48, 53, 56, 60 °C), as shown in Tables 4 and 5. Hence, lactate production from decayed sludge components becomes significant under the incubation at 50 °C.

3.4. Dynamics of culturable viable bacteria and lactic acid bacteria during lactate fermentation

We examined the dynamics of culturable viable bacteria and lactate bacteria during lactic acid fermentation to reveal the reason why the fermentation at 50 °C led to high conversion rate to lactic acid. Both total viable bacteria and lactic acid bacteria increased with time in the fermentation at 30 and 40 °C, although they decreased at 60 °C. On the other hand, at 50 °C, lactic acid bacteria increased for 1 d whereas total viable bacteria did not increased; after 1-d incubation, both bacteria decreased (Fig. 2). These results indicate that in the fermentation at 50 °C sucrose and excess sludge were preferentially utilized to produce lactate by the sucrose and excess sludge rather than for the cell synthesis (growth) of the total population of viable bacteria. Also, lactic acid bacteria were not able to survive at 60 °C in agreement with the results that lactic acid was not produced in the fermentation at 60 °C (Table 4 and Fig. 2). Also, 33 clones (from samples after 1 d at 50 °C) grown along with any haloes were tested randomly by API 50CHL to confirm whether these clones are lactic acid bacteria. The results of API 50CHL showed that 12 clones were *Lacto. acidophilus* 1 (98.9%), 6 clones were *Lacto. delb.delb* (70.9%), 5 clones were *Lacto acidophilus* 3 (98.1%), 2 clones were *Lc. lactis lactis* 1 (99.6%), and 2 clones were *Lacto. delb.delb* (81.3%) and the remaining clones (each 1 clone) were *Lc. raffinolactis* (96.5%), *Weis. viridescens* (96.8%), *Lc. lactic lactis* 1 (96.5%), *Lacto. acidophilus* 1 (79.8%), *Lc. lactic lactis* 1 (77.9%), and *Lacto. acidophilus* 1 (71.2%); hence, it indicated clearly that almost all the clones were lactic acid bacteria.

3.5. pH-vibration fermentation for enhanced lactic acid production

We performed pH-vibration fermentation at 50 °C for completely consuming sucrose and for producing lactic acid maximally,

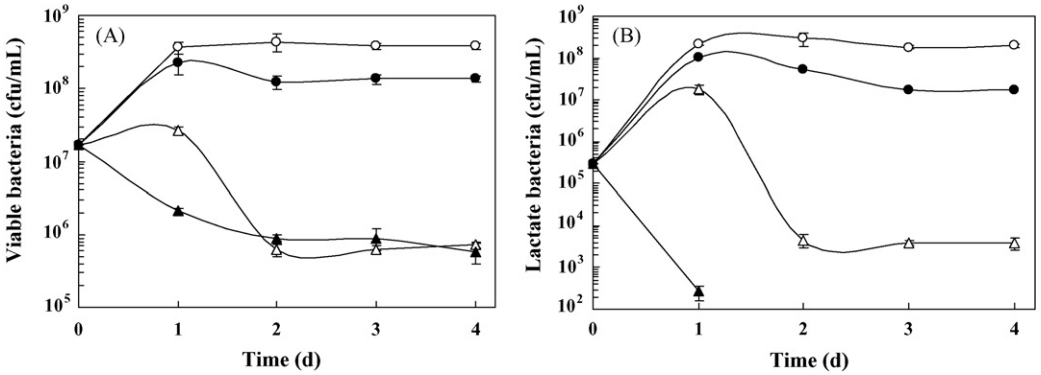

Fig. 2. Dynamics of viable bacteria (A) and lactic acid bacteria (B) during lactic acid fermentation at 30 °C (○), 40 °C (●), 50 °C (△), and 60 °C (▲) by using excess sludge containing 50 mM sucrose. Standard deviations are shown from two independent experiments.

since a little over half of the sucrose concentration remained without consuming completely in the standard fermentation at 50 °C. The pH value in excess sludge was adjusted to 6.8 for every 1 d by using 25% aqueous ammonia, as shown in Fig. 3A. The pH-vibration fermentation resulted in the complete consumption of sucrose concomitantly with highly producing lactic acid (Fig. 3B) compared to the standard fermentation. Lactic acid was preferentially produced by the pH-vibration fermentation until 2–3 d although it was gradually consumed after 3 d, as shown in Fig. 3B. After the decrease of lactic acid, butyric acid and isobutyric acid increased with time (Fig. 3C). As corroborating evidence, the number of lactate bacteria increased with time whereas the total number of viable bacteria decreased gradually after 1-d cultivation (Fig. 3D). Also, the pH-vibration fermentation led to enhanced sludge reduction in comparison with the standard fermentation (Fig. 4A). Conversion rates to lactic acid increased up to 120% by the pH-vibration fermentation, although they decreased after 3 d according to the decrease of lactic acid (Fig. 4B).

3.6. Mechanism of enhanced lactate production and sludge reduction

To characterize the mechanism of the excess sludge reduction, the effect of temperature, bacterial activity, and enzyme activity (amylase and cellulase) was tested. The reduction of excess sludge was due to the temperature effect and sludge-lysing activity present in excess sludge since sludge-reduction rate at 50 °C was higher than that at 30 °C and since the fermentation at pH 3.2 initially led to a significant decrease of sludge reduction (Fig. 5), as well as the results obtained by using propionic acid, formic acid, and hydrochloric acid (data not shown). Also, the amylase and cellulase activity in the supernatant of excess sludge incubated at 50 °C for 1 d were 2.6 ± 0.5 μmol-maltose/mg-protein/h from 1% starch and 1.0 ± 0.1 μmol-glucose/mg-protein/h from 2% carboxylmethyl cellulose.

Furthermore, to understand the mechanism of enhanced lactate production and sludge reduction more clearly, the lactate fermen-

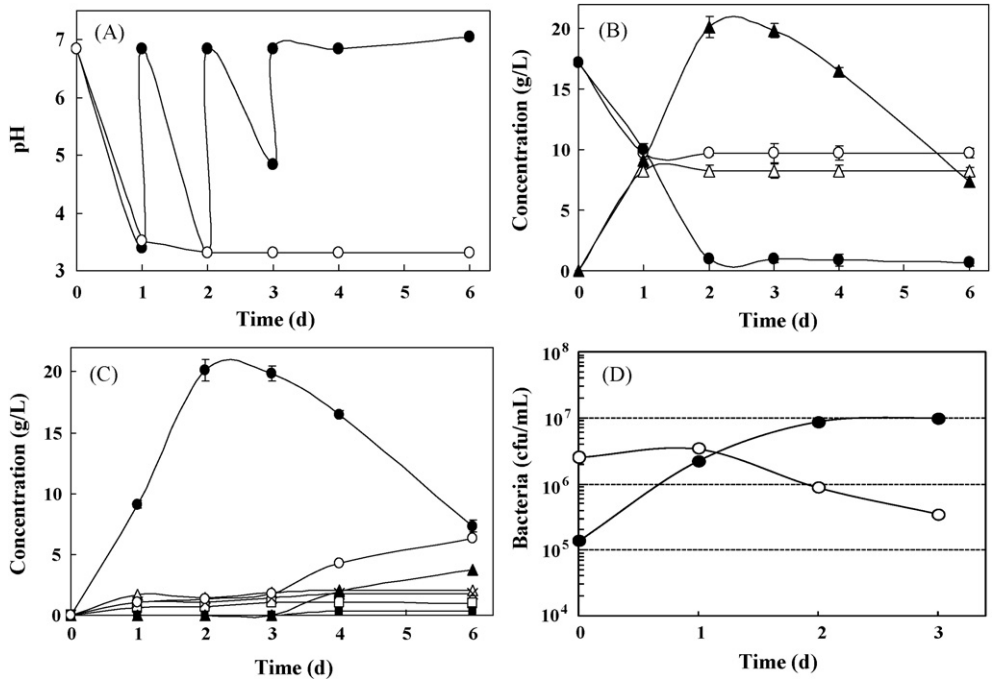

Fig. 3. pH-vibration fermentation at 50 °C by excess sludge containing 50 mM sucrose. (A) pH change during the standard fermentation (open) and pH-vibration fermentation (solid). (B) Sucrose (circle) and lactic acid concentration (triangle) during standard (open) and pH-vibration fermentation (solid). (C) Dynamics of organic acid produced by pH-vibration fermentation from excess sludge containing 50 mM sucrose. Lactic acid (●), succinic acid (△), formic acid (□), acetic acid (×), propionic acid (■), butyric acid (○), and isobutyric acid (▲) were measured by HPLC. (D) The number of viable bacteria (○) and lactic acid bacteria (●) during pH-vibration fermentation. Standard deviations are shown from two independent experiments.

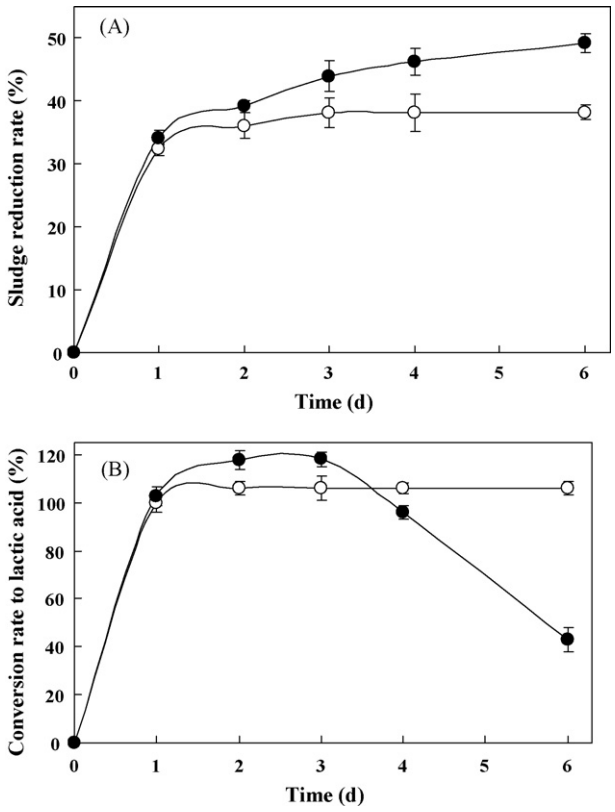

Fig. 4. Sludge reduction (A) and conversion rate from sucrose to lactic acid (B) in pH-vibration fermentation (●) and standard fermentation (○) at 50 °C by using excess sludge containing 50 mM sucrose. Standard deviations are shown from three independent experiments.

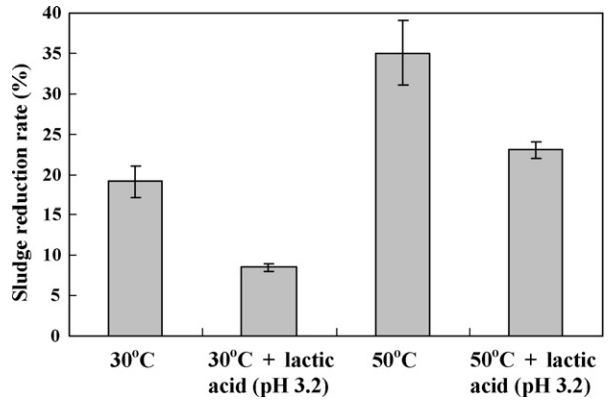

Fig. 5. Excess sludge reduction at 30 °C and 50 °C with or without adding lactic acid initially. Standard deviations are shown from three independent experiments.

tation experiment using a purely cultured *Lacto. acidophilus* strain TS1 which was one of the 12 clones isolated preferentially from the fermented samples for 1 d at 50 °C was performed by using autoclaved sludge containing 50 mM sucrose. As shown in Table 6, about 20% of excess sludge was reduced through the autoclave treatment, resulting in a 5-fold and 93-fold increased carbohydrate and protein concentration in the supernatant of autoclaved excess sludge compared to those of non-autoclaved excess sludge. Furthermore, lactic acid production from the mixture of the autoclaved excess sludge and sucrose (final concentration 50 mM) by lactic acid bacterium strain TS1 reached to 5.59 ± 0.03 g/L by the fermentation at 50 °C for 4 d; in addition, the conversion rate to lactic acid and sludge-reduction rate were 113% and 5%, respectively. These results indicated clearly that the isolated lactate bacteria can produce lactic acid from protein and carbohydrate contents derived from excess sludge and that sludge reduction will be due to other factors such as temperature and sludge-lysing bacteria rather than due to the lactic acid bacteria. Also, since the results of Fig. 5 implied that there were sludge-lysing bacteria in excess sludge, the screening of sludge-lysing bacteria from excess sludge at 50 °C was conducted. As a result, two clones (named KH3 and KH4) that formed a sludge-lysing halo on the plates were isolated (data not shown); KH3 and KH4 could reduce 33 ± 1% and 23 ± 3% of autoclaved excess sludge, along with an increased protein and carbohydrate concentration in the supernatant of excess sludge (Fig. 6). Taken together, it appears that the reason of the phenomenon that lactate fermentation with excess sludge containing sucrose at 50 °C had higher lactic acid production than theoretical value is due to the collaboration with sludge-reduction processes (e.g. temperature and sludge-lysing bacteria) and specially adapted lactate bacteria such as strain TS1.

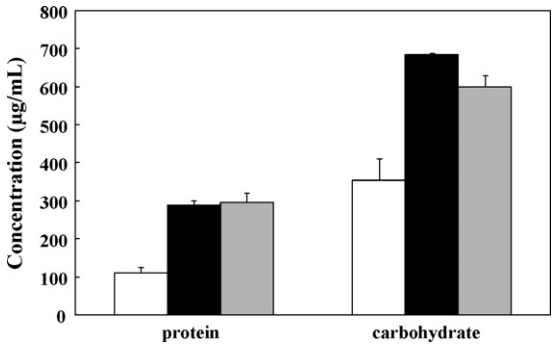

Fig. 6. Protein and carbohydrate contents in the supernatant of excess sludge reacted with KH3 (black bars) and KH4 (gray bars) for 5 d at 50 °C. White bars are controls without the inoculation of bacteria. Standard deviations are shown from two independent experiments.

4. Discussion

Constructing a facile engineering for effectively recycling and reducing excess sludge is required as one of the environmentally friendly technologies. In this paper, we performed lactic acid fermentation at 50 °C to produce lactic acid, which is a beneficial compound, from excess sludge containing sucrose and found that conversion rate from sucrose to lactic acid was more than 100% according to the reduction of excess sludge, indicating that a part of excess sludge was used as a material for producing lactic acid during the fermentation. Our results clearly showed that (1) lactate fermentation with autoclaved excess sludge containing sucrose by purely cultured lactate bacteria present in excess sludge had more than 100% of lactate conversion rate, (2) temperature and

Table 6
Total solid, protein concentration, and carbohydrate concentration in the supernatant of excess sludge before and after autoclave for 20 min at 121 °C.

	Total solids		Protein concentration		Carbohydrate concentration	
	mg-dry/g-wet	Relative	μg/mL	Relative	μg/mL	Relative
Before	24 ± 1	1	33 ± 35	1	355 ± 0	1
After	19 ± 1	0.8	2783 ± 165	84	1813 ± 396	5

bacterial sludge-lysing activity such as sludge-lysing bacteria, amylase, and cellulase were responsible for the reduction of excess sludge, and (3) there were actually sludge-lysing bacteria in excess sludge. Therefore, it appears that the mechanism by which lactic acid production from excess sludge containing sucrose had higher than theoretical value is that specially adapted lactic acid bacteria were able to produce lactic acid from proteins and carbohydrates released from excess sludge as well as from sucrose. Since it has been reported that cysteine, tryptophan, and serine among amino acids convert into pyruvate [43], which is a good substrate to produce lactic acid [44], the proteins are one of the suitable substrates for producing lactic acid. Other factors contributing to the observed sludge reduction are the amylase and cellulase activity because the supernatant of excess sludge actually includes these two enzymes which are related to produce monosaccharide and/or disaccharide from starch and cellulose; hence, sugar contents produced from starch and cellulose by these enzyme activities should be used for producing lactic acid.

To have more than 100% of conversion rate from sucrose to lactic acid, the strict temperature condition at 50 °C was required; our results showed that (1) culturable viable bacteria and lactate bacteria had a low activity in the lactate fermentation at 60 °C, that (2) the number of viable bacteria and lactate bacteria at 50 °C and 60 °C decreased after at least 2 d although they did not decreased at 30 °C and 40 °C, and that (3) in the pH-vibration fermentation, the number of lactic acid bacteria certainly increased with time although the number of total viable bacteria gradually decreased. These results implied that lactic acid bacteria present in excess sludge had still an enough lactate-producing activity at 50 °C (Note that the decrease of the number of lactic acid bacteria in the standard fermentation at 50 °C will be due to just the decrease of pH not temperature effect although in the fermentation at more than 60 °C, the number of lactic acid bacteria will be decreased by temperature effect) whereas viable bacteria have a low bacterial activity due to both the temperature effect (at more than 50 °C) and low pH; therefore, our results that lactate fermentation at 50 °C by using excess sludge containing sucrose had more than 100% of conversion rate to lactic acid appears to be due to the complex bacterial dynamics between lactic acid bacteria and other viable bacteria (it mainly means lactate production from sucrose by lactic acid bacteria vs. cell synthesis from sucrose by other viable bacteria). In addition, the phenomenon that conversion rate to lactic acid was more than 100% at 50 °C may indicate that there are specific lactate bacteria which can produce 2 mol of lactic acid from 1 mol of sugar (i.e. homolactic fermentation) in the excess sludge at 50 °C and that they are operative to consume sugar and/or protein contents for producing lactic acid in preference to other types of lactate bacteria (for example, heterolactic fermentation) and other viable bacteria. Also, the fermentation at 50 °C with sucrose showed an effective lactic acid production compared to that by other sugars such as glucose; the reason may be due to an increased catabolic inefficiency of microbes by sucrose, reported as previously [45]. On the other hand, pH-vibration fermentation allows the complete sucrose consumption and maximizes the lactic acid production by controlling such a bacterial activity in excess sludge.

In this study, total 33 clones of lactic acid bacteria were isolated from excess sludge by using our screening method; the results of API50CHL proved that almost all the clones were lactic acid bacteria, as corroborated by the HPLC analyses indicating that lactic acid was principally produced by the fermentation with excess sludge. Although a developed selective medium for the isolation of lactic acid bacteria has been used [46], our screening method able to check organic acid-producing bacteria by the formation of a clear zone may be useful as an alternative way of the screening. On the other hand, the two sludge-lysing bacteria, KH3 and KH4 were isolated from excess sludge at 50 °C; how KH3 and KH4 are able to reduce excess sludge is an interesting topic. Our latest results on these bacteria showed that both KH3 and KH4 have protease activity which appears to be related to the reduction of excess sludge in a previous study [16]. Also, the effect of KH3 for excess sludge reduction was recently reported [47].

So far, a facile engineering for reducing and recycling excess sludge has been strongly required since a large quantity of excess sludge has been formed every day. To date, there are many reports on the excess sludge reduction; in particular, although current sludge-reducing methods based on physical and chemical treatment such as ultrasonic and ozone treatments have high sludge-reduction ability, a great deal of energy will be required for these treatments [48]. Also, how to utilize the protein and carbohydrate contents released from excess sludge by the physical treatments will be the next issue. On the other hand, our study using lactic acid fermentation is a favorable technique as one of the facile technologies because our method is simultaneously applicable both to reduce excess sludge and to produce a beneficial product although there is a serious problem that lactate fermentation could not proceed without adding sucrose in excess sludge.

5. Conclusion

Lactic acid fermentation using excess sludge containing sucrose showed more than 100% of lactic acid conversion rate concomitantly with enhanced excess sludge reduction, indicating that a part of decayed excess sludge was utilized to produce lactic acid. The phenomenon was specific to the incubation at 50 °C due to the preferential activity of lactic acid bacteria. The pH-vibration fermentation enhanced lactic acid production and excess sludge reduction. Taken together, it was concluded that our finding was due to the collaboration with sludge-reduction processes and specially adapted lactate bacteria.

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