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Lactic acid production from agricultural resources as cheap raw materials

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Abstract

Agricultural resources such as barley, wheat, and corn were hydrolyzed by commercial amylolytic enzymes and fermented into lactic acid by *Enterococcus faecalis* RKY1. Although no additional nutrients were supplemented to those resources, lactic acid productivities were obtained at >0.8 g/l h from barley and wheat. When 200 g/l of whole wheat flour was hydrolyzed by amylolytic enzymes after the pre-treatment with 0.3% (v/v) sulfuric acid and sterilized by filtration, *E. faecalis* RKY1 efficiently produced lactic acid with 2.6 g/l h of lactic acid productivity and 5.90 g/l of maximal dry cell weight without additional nutrients. Lactic acid productivity and cell growth could be enhanced to 31% and 12% higher values than those of non-adapted RKY1, by adaptation of *E. faecalis* RKY1 to CSL-based medium. When the medium contained 200 g/l of whole wheat flour hydrolyzate, 15 g/l of corn steep liquor, and 1.5 g/l of yeast extract, lactic acid productivity and maximal dry cell weight were obtained at 5.36 g/l h and 14.08 g/l, respectively. This result represented an improvement of up to 106% of lactic acid productivity and 138% of maximal dry cell weight in comparison to the fermentation from whole wheat flour hydrolyzate only.

Keywords: Lactic acid; Agricultural resources; Corn steep liquor; Submerged fermentation; *Enterococcus faecalis*

1. Introduction

Lactic acid has various applications in food, pharmaceutical, leather, and textile industries (VickRoy, 1985). Since lactic acid has high reactivity due to containing both hydroxyl (–OH) and carboxyl (–COOH) groups, it plays a major role as a chemical feedstock capable of being converted to various chemicals such as acrylic acid, propylene glycol, acetaldehyde, and 2,3-pentanedione (Varadarajan and Miller, 1999). The continuous increase in demand for lactic acid has been due to its increasing applications in preparation of biodegradable polymers, medical sutures, and green solvents (Datta et al., 1995; Litchfield, 1996). Lactic acid is industrially produced either by chemical synthesis or by microbial fermentation. A biological method has the advantage that an optically pure lactic acid can be obtained by choosing a strain of lactic acid bacteria, whereas chemical synthesis always results in a racemic mixture of lactic acid (Ryu et al., 2003). The presence of L(+)-lactic acid with high optical purity gives polylactic acids of high melting point and high crystallinity (Lunt, 1998; Yun and Ryu, 2001).

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Lactic acid bacteria are traditionally fastidious microorganisms and have complex nutrient requirements due to their limited ability to biosynthesize B-vitamins and amino acids (Fitzpatrick and O'Keeffe, 2001). Refined sugars such as glucose or sucrose have been more frequently used to produce lactic acid than raw starchy substrates such as barley, corn, or wheat (Hofvendahl and Hahn-Hägerdal, 1997). Furthermore, a considerable amount of expensive complex nitrogen source, such as yeast extract, must be added to the medium to produce lactic acid in a reasonable time. However, these are economically unfavorable because pure sugars and pure complex nitrogen sources are expensive but lactic acid is a relatively cheap product. Therefore, raw materials for industrial lactic acid production need to have several characteristics such as low cost, low levels of contaminants, rapid fermentation rate, high lactic acid yields, little or no by-product formation, and year-round availability (Ryu et al., 2003). According to Tejayadi and Cheryan (1995), the cost for raw material possessed 68% of the total cost for lactic acid production from whey permeate and yeast extract using *Lactobacillus* (*Lb.*) *bulgaricus*. Åkerberg and Zacchi (2000) also previously reported, through the simulation study of lactic acid fermentation process, that the operational cost including raw material, neutralizing agent, hydrolyzing enzyme, and membrane for electrodialysis possessed approximately 80% of the total cost for lactic acid production from wheat flour. Since raw material cost cannot be reduced by scaling-up the process, starchy material and/or corn steep liquor have been considered as attractive nutrient sources for industrial lactic acid production.

We report here the lactic acid production from agricultural and renewable resources (such as barley, corn, and wheat) as cheap raw substrates without additional nutrients. We also evaluated the effect of pre-treatment methods of agricultural resource, which can release fermentable glucose, on lactic acid production. The addition of corn steep liquor (CSL) was investigated to improve the lactic acid fermentations. Furthermore, we investigated the effect of cell adaptation to CSL-based medium on lactic acid fermentations.

2. Methods

2.1. Strain and growth medium

Enterococcus faecalis RKY1 (Yun and Ryu, 2001; Ryu et al., 1999, 2001; Oh et al., 2003), a homofermentative L(+)-lactic acid producer, was utilized in this work. Stock cultures were maintained at −20 °C in 5 ml vials containing 50% (v/v) glycerol until used. Unless otherwise mentioned, the medium for cell growth contained the followings (g/l): glucose 30, yeast extract 10, and K_2HPO_4 5. The growth medium for adaptation culture to CSL-based medium was composed of 30 g/l of glucose, 10 g/l of CSL (solid basis), 3 g/l of yeast extract, and 5 g/l of K_2HPO_4. Yeast extract was obtained from Difco Laboratories (Detroit, MI), glucose and K_2HPO_4 were purchased from Yakuri Chemicals Co. (Tokyo, Japan), and CSL was kindly offered from TS Corporation Research and Development Center (Incheon, Korea). The pH was adjusted to 7.0 prior to sterilization at 121 °C for 15 min.

2.2. Enzymatic hydrolysis

The agricultural resources such as barley, wheat, and corn were kindly provided by TS Corporation Research and Development Center, and they were minutely grinded by milling process. Up to 250 g of milled flour was suspended in 800 ml of tap water and the pH of this suspension was adjusted to 6.0. Three hundred microgram of α-amylase, Termamyl 120L, Type LS (Novo Nordisk A/S, Bagsvaerd, Denmark), was added to the suspension and then the mixture was heated to optimal temperature for enzymatic liquefaction at 95 °C for 30 min. The liquefied solution was cooled to room temperature, and then the pH was re-adjusted to 4.5. After the temperature of the liquefied solution reached below 60 °C, 300 μg of glucoamylase, AMG 300L (Novo Nordisk A/S) was added. This saccharification step was aseptically performed on a sterilized 2 l Erlenmeyer flask at 55 °C and 200 rpm for 24 h. The saccharified solution was filtrated through 2 μm filter paper, which was then used for main fermentation medium. If necessary, some other additives such as CSL and/or yeast extract were added to the saccharified solution.

2.3. Inoculum preparation and batch fermentation

E. faecalis RKY1 cells from stock cultures were transferred to 15 ml sterile growth medium in a 20 ml vial and incubated at 38 °C for 10 h. A 0.6 ml of this culture was then transferred to a new growth medium (15 ml) in a 20 ml vial every 10 h. After 4–5 consecutive propagation steps, 3 ml of this culture was then transferred to a 50 ml vial that contained 40 ml growth medium. The inoculum was incubated at 38 °C for 6 h on a shaking incubator (KMC-8480SF, Vision Scientific Co., Daejeon, Korea) at 200 rpm before inoculation at 4% (v/v) to the fermenter. Batch fermentations were performed on a KF-2.5L fermenter (Kobiotech Co., Incheon, Korea) containing 1 l working volume and controlled at 38 °C and 200 rpm. The culture pH during batch fermentations was maintained at 7.0 by automatic addition of 10 N NaOH. The samples were aseptically withdrawn at desired intervals and were frozen at −20 °C for further analysis.

2.4. Analyses

Lactic acid was analyzed by a high-performance liquid chromatography (HPLC) system (Waters, Millipore Co., Milford, MA) equipped with Waters 486 tunable absorbance detector set to 210 nm. An Aminex HPX-87H ion-exclusion column (300 × 7.8 mm, Bio-Rad, Hercules, CA) was used with 0.008 N H_2SO_4 as mobile phase at a flow rate of 0.6 ml/min, while the column temperature was maintained at 35 °C. Glucose was enzymatically determined with a Glucose-E kit (YD Diagnostics, Seoul, Korea) through glucose oxidase–peroxidase method. Cell concentration was measured with a spectrophotometer (UV-160A, Shimadzu Co., Tokyo, Japan) at a wavelength of 660 nm. Dry cell weight was calculated through a standard curve relating the optical density at 660 nm to dry weight (g/l). One unit of optical density at 660 nm corresponded to a 0.8 g-dry cell weight/l. All the experiments were carried out in duplicate and the mean values are reported.

3. Results and discussion

3.1. Lactic acid production from agricultural resources without any supplementations

Lactic acid fermentations from agricultural resources such as barley, wheat, and corn flours were performed on a laboratory-scale bioreactor without any supplementations in order to investigate the possibility of those raw materials as a sole nutrient source. Table 1 shows the results of lactic acid production when the medium was composed of each saccharified liquor from 200 g of agricultural resources without any supplementations. The volumetric productivities of lactic acid and maximal dry cell weight reached to 0.51–0.88 g/l h and 1.67–2.25 g/l, respectively, where barley was found to be the most efficient nutrient among three resources tested. On the other hand, glucose yield after liquefaction and saccharification was highest at corn flour. Lactic acid yields based on consumed glucose were above 0.92 g/g in all the cases experimented. Javanainen and Linko (1995) previously reported that 36 g/l of lactic acid (0.75 g/l h) was produced from 180 g of barley flour (equivalent to 119 g of glucose) as a single nutrient source after 48 h of mixed culture of *Lb. amylovorus* and *Lb. casei* and 120 g/l of lactic acid (0.83 g/l h) was produced by simultaneous saccharification and fermentation through the addition of glucoamylase after 144 h of fermentation. We obtained 133 g of glucose from 200 g of barley flour through enzymatic hydrolysis, from which 38 g/l of lactic acid was produced and 2.3 g/l of maximal dry cell weight was obtained after 43 h of fermentation. This result is quite similar to those of Javanainen and Linko (1995).

In order to confirm the effect of pre-treatment methods of barley flour on lactic acid fermentations, we pre-treated barley flour through sulfuric acid treatment, and the results are shown in Table 2. Sulfuric acid treatment generally enhanced the glucose yield and fermentation efficiencies, and lactic acid productivities increased with increasing sulfuric acid concentration used for pre-treatment. When 0.5% (v/v) sulfuric acid was used for pre-treatment of 150 g barley flour, lactic acid productivity and maximal dry cell weight were approximately 2 times higher than those of non-sulfuric acid treatment. Hsiesh et al. (1999) reported that, when soy protein hydrolyzates with several molecular weights were used for lactic acid fermentations, lactic acid production was improved by decreasing soy peptide molecular weight from >10,000 Da to 700 Da. Therefore, *E. faecalis* RKY1 cells might easily uptake nitrogen source because protein moieties of barley flour were fragmented adequately for cell growth by sulfuric acid pre-treatment.

We also investigated the effect of sterilization methods of barley flour hydrolyzate on lactic acid fermentations (Table 2). Two sterilization methods, autoclaving at 121 °C (15 psi) and filtration through hollow fiber filter (SKUF-103-0830, SK Chemical Co., Suwon, Korea) with 30 kDa molecular weight cut-off, were experimented. When 0.3% (v/v) sulfuric acid treatment and sterilization by filtration were simultaneously introduced, although sulfuric acid concentration used for pre-treatment of 150 g barley flour was lower than that of 0.5% (v/v) sulfuric acid treatment, lactic acid productivity and maximal dry cell weight increased to 2.6 g/l h and 5.90 g/l (2.28 g/l h and 3.56 g/l at 0.5% sulfuric acid treatment), respectively. These results imply that autoclaving at high temperature and high pressure under

Table 1
Lactic acid fermentations from agricultural resource as a sole nutrient by batch cultivation of *E. faecalis* RKY1

Agricultural resources (200 g)	Glucose yield[a] (%)	Lactic acid yield[b] (g/g)	Lactic acid productivity (g/l h)	Maximal dry cell weight (g/l)
Barley	65.0 ± 1.7	0.94 ± 0.02	0.88 ± 0.02	2.29 ± 0.11
Wheat	52.5 ± 1.4	0.93 ± 0.01	0.81 ± 0.02	1.67 ± 0.08
Corn	67.5 ± 1.5	0.94 ± 0.01	0.51 ± 0.01	1.75 ± 0.10

Batch fermentations were performed on a 2.5 l jar fermenter with 1 l working volume at pH 7.0, 38 °C, and 200 rpm. Data are presented as the means of two replicates and ± denotes standard deviation among the replicates.

[a] g-Glucose released/g-agricultural resources × 100.

[b] g-Lactic acid produced/g-glucose consumed.

Table 2
Effect of pre-treatment and sterilization methods on lactic acid fermentations using whole barley flour as a sole nutrient source

Barley flour (g/l)	Pre-treatment	Sterilization	Lactic acid yield[a] (g/g)	Lactic acid productivity (g/l h)	Maximal dry cell weight (g/l)
100	None	Autoclaving	0.92 ± 0.01	0.98 ± 0.04	1.89 ± 0.07
100	0.3% (v/v) H_2SO_4	Autoclaving	0.94 ± 0.01	1.03 ± 0.02	2.35 ± 0.12
150	None	Autoclaving	0.93 ± 0.02	1.16 ± 0.05	1.13 ± 0.08
150	0.5% (v/v) H_2SO_4	Autoclaving	0.94 ± 0.02	2.28 ± 0.06	3.56 ± 0.20
150	0.3% (v/v) H_2SO_4	Filtration	0.93 ± 0.01	2.60 ± 0.04	5.90 ± 0.23
200	None	Autoclaving	0.94 ± 0.01	0.88 ± 0.02	2.29 ± 0.11

Batch fermentations were performed on a 2.5 l jar fermenter with 1 l working volume at pH 7.0, 38 °C, and 200 rpm. Data are presented as the means of two replicates and ± denotes standard deviation among the replicates.
[a] g-Lactic acid produced/g-glucose consumed.

acidic conditions lead to formation of inhibiting compounds (e.g. 5-hydroxymethyl furfural derived from glucose degradation) and liberation of some nutrient sources (Palmqvist and Hahn-Hägerdal, 2000). However, lactic acid yields based on consumed glucose were between 0.92 g/g and 0.94 g/g in both cases experimented. Therefore, we concluded that, in lactic acid fermentations from agricultural resources hydrolyzate, the sterilization method by filtration was more favorable than by autoclaving.

3.2. Lactic acid production from whole wheat flour with corn steep liquor

In order to investigate the effect of cell adaptation to CSL-based medium on lactic acid fermentations, *E. faecalis* RKY1 was grown on the medium containing CSL for 15 days. Table 3 shows the substrate conversion ratio, lactic acid production, lactic acid yield, maximal dry cell weight, and lactic acid productivity in adapted (CSL-based medium) and non-adapted (yeast extract-based medium) *E. faecalis* RKY1. When the strain RKY1 was adapted to CSL-based medium, the fermentation could be completed 6 h faster than non-adaptation. Lactic acid productivity and maximal dry cell weight increased to 31% and 12% higher than those of non-adapted RKY1 by adaptation to CSL-based medium. For further study, therefore, *E. faecalis* RKY1 was adapted to CSL-based medium before the fermentations.

To improve the economics of lactic acid fermentation, CSL as a cheap nitrogen source was tested using whole wheat flour hydrolyzate. Corn steep liquor was added to wheat flour hydrolyzate at initial concentrations of 0, 5, 10, 15, and 20 g/l (solid basis) for investigation of the effect of CSL concentrations on lactic acid fermentations. As shown in Fig. 1, cell concentration of *E. faecalis* RKY1 increased with CSL supplementation up to 10 g/l, but there was no significant improvement in cell concentration beyond that value. Lactic acid production was also enhanced by increasing CSL supplementations. When wheat flour hydrolyzate was supplemented with CSL from 0 g/l to 25 g/l, lactic acid productivity increased from 1.21 g/l h to 4.14 g/l h (Table 4). Lactic acid yields based on consumed glucose were between 0.92 g/g and 0.94 g/g in all the cases experimented. Although CSL is a by-product of corn steeping process, CSL supplementation had a beneficial effect on lactic acid production, glucose utilization, and cell growth during fermentation. Complex nitrogen source, especially yeast extract, is the most frequently used for lactic acid fermentations because it offers rapid

Table 3
Effect of growth medium on lactic acid fermentations from whole wheat flour hydrolyzate and CSL

Growth medium	Fermentation time (h)	Substrate conversion[a] (%)	Lactic acid (g/l)	Lactic acid yield[b] (g/g)	Lactic acid productivity (g/l h)	Maximal cell growth (g/l)
Yeast extract-based medium[c] (before adaptation)	33	93.4 ± 0.8	95.5 ± 0.5	0.94 ± 0.02	2.89 ± 0.01	9.90 ± 0.22
CSL-based medium[d] (after adaptation)	27	99.0 ± 0.9	102.7 ± 1.6	0.93 ± 0.01	3.80 ± 0.06	11.07 ± 0.06

Batch fermentations were performed on a 2.5 l jar fermenter with 1 l working volume at pH 7.0, 38 °C, and 200 rpm. Data are presented as the means of two replicates and ± denotes standard deviation among the replicates.
[a] g-Glucose consumed/g-initial glucose × 100.
[b] g-Lactic acid produced/g-glucose consumed.
[c] Yeast extract-based medium was composed of 30 g/l of glucose, 10 g/l of yeast extract, and 5 g/l of K_2HPO_4.
[d] CSL-based medium was composed of 30 g/l of glucose, 10 g/l of CSL, 3 g/l of yeast extract, and 5 g/l of K_2HPO_4.

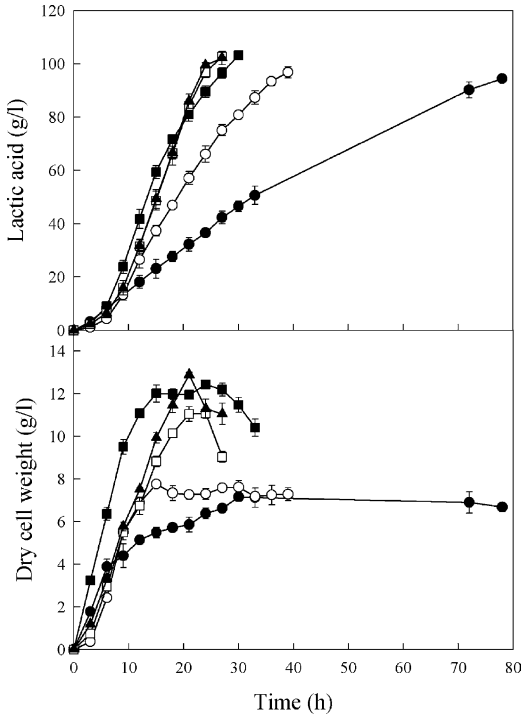

Fig. 1. Profiles of lactic acid fermentation from whole wheat flour hydrolyzate and CSL by batch fermentation of *E. faecalis* RKY1. The medium containing 200 g/l of whole wheat flour hydrolyzate and 0–25 g/l of CSL was sterilized by filtration. Data are presented as the means of two replicates and error bars indicate the standard deviation. (●) CSL 0 g/l; (○) CSL 5 g/l; (■) CSL 10 g/l; (□) CSL 15 g/l; (▲) CSL 25 g/l.

production and high productivity of lactic acid. The high cost of yeast extract, however, has a negative impact on the economics of its use in commercial processes. Although the nitrogen content of CSL is dependent upon steeping processes chosen, it is derived from corn itself because 85% of total nitrogen is composed of protein, peptide, and free amino acid (Dailey et al., 2000).

When 1.5 g/l of yeast extract (about 1.5% to glucose content in wheat flour hydrolyzate) was supplemented to the medium containing 200 g/l of wheat flour hydrolyzate and 15 g/l of CSL, maximal dry cell weight and lactic acid productivity were obtained as 14.08 g/l and 5.36 g/l h, respectively (Fig. 2), which were 27% and 41% higher values than those of lactic acid fermentation using wheat flour hydrolyzate without yeast extract. These results show that the fermentation efficiencies (e.g. cell growth and productivity) could be significantly increased by addition of trace amount of yeast extract (1.5% to saccharified glucose) to the cheap medium containing wheat flour hydrolyzate and CSL.

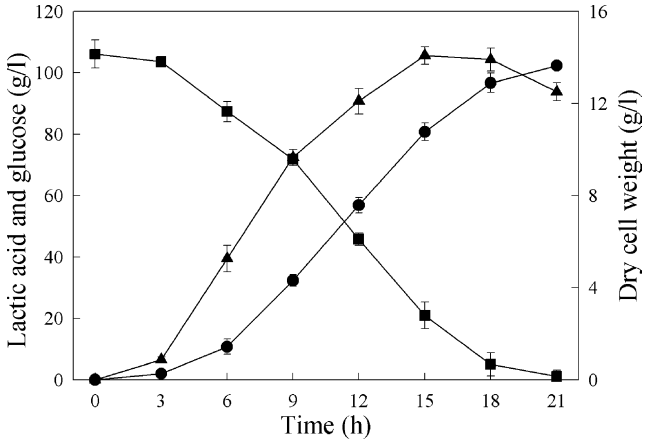

Fig. 2. Lactic acid fermentation from 200 g/l of whole wheat flour hydrolyzate supplemented with 15 g/l of CSL and 1.5 g/l of yeast extract. Data are presented as the means of two replicates and error bars indicate the standard deviation. (●) lactic acid; (■) glucose; (▲) dry cell weight.

There are two types of fermentation for the production of biochemicals from agricultural resources, i.e. submerged fermentation (SmF) and solid-state fermentation (SSF). Lactic acid can be produced both by SmF and SSF, but our results reported here were obtained from SmF. Table 5 shows the results from this work and from several literatures previously reported about lactic acid production from agricultural resources using SmF or SSF. According to Soccol et al. (1994), although lactic acid yields were 0.77 g/g whatever was the medium, lactic acid was obtained as 93.8 g/l and

Table 4
Effect of CSL concentrations on lactic acid fermentations using whole wheat flour hydrolyzate by batch cultivation of *E. faecalis* RKY1

CSL (g/l)	Fermentation time (h)	Substrate conversion[a] (%)	Lactic acid (g/l)	Lactic acid yield[b] (g/g)	Lactic acid productivity (g/l h)	Maximal dry cell weight (g/l)
0	78	96.7 ± 0.5	94.4 ± 0.8	0.94 ± 0.01	1.21 ± 0.01	7.15 ± 0.05
5	39	96.9 ± 0.8	96.8 ± 2.1	0.92 ± 0.02	2.48 ± 0.06	7.75 ± 0.12
10	30	96.9 ± 1.0	103.1 ± 1.1	0.94 ± 0.01	3.43 ± 0.03	12.42 ± 0.09
15	27	99.0 ± 1.2	102.7 ± 1.6	0.93 ± 0.01	3.80 ± 0.06	11.07 ± 0.15
25	24	98.9 ± 0.9	99.4 ± 1.0	0.92 ± 0.01	4.14 ± 0.06	12.8 ± 0.11

Batch fermentations were performed on a 2.5 l jar fermenter with 1 l working volume at pH 7.0, 38 °C, and 200 rpm, and the medium containing 200 g/l of wheat hydrolyzate and 0–25 g/l of CSL was sterilized by filtration. Data are presented as the means of two replicates and ± denotes standard deviation among the replicates.

[a] g-Glucose consumed/g-initial glucose × 100.

[b] g-Lactic acid produced/g-glucose consumed.

Table 5
Data reported on batch fermentations for lactic acid production from agricultural resources

Microorganisms	Fermentation type	Raw material	Lactic acid (g/l)	Lactic acid yield[a] (g/g)	Productivity (g/l h)	References
Rhizopus oryzae	SmF	Sugar-cane bagasse	93.8	0.77	1.38	Soccol et al. (1994)
Rhizopus oryzae	SSF	Sugar-cane bagasse	137.0	0.77	1.43	Soccol et al. (1994)
Lactobacillus paracasei	SmF	Sweet sorghum	88–106	0.91–0.95	3.31–3.67	Richter and Träger (1994)
Lactobacillus paracasei	SSF	Sweet sorghum	90[b]	0.91–0.95	0.45–0.75[c]	Richter and Träger (1994)
Lactobacillus delbrueckii	SmF	Whole wheat	106	0.95	1.00	Hofvendahl and Hahn-Hägerdal (1997)
Lactococcus lactis	SmF	Whole wheat	109	0.93	1.09	Hofvendahl and Hahn-Hägerdal (1997)
Enterococcus faecalis RKY1	SmF	Whole wheat	102	0.97	4.87	This work

SmF and SSF represent submerged fermentation and solid-state fermentation, respectively.
[a] g-Lactic acid produced/g-sugars consumed.
[b] g-Lactic acid/kg-raw material.
[c] g-Lactic acid/kg-raw material h.

137 g/l in SmF and SSF, respectively, by batch culture of *Rhizopus oryzae*. Lactic acid productivities were 1.38 g/l h and 1.43 g/l h in SmF and SSF, respectively. Richter and Träger (1994) reported that, when *Lb. paracasei* was used for lactic acid production from sweet sorghum, lactic acid concentrations and yields were 88–106 g/l and 0.91–0.95 g/g for SmF and 90 g/kg and 0.91–0.95 g/g for SSF, respectively. Hofvendahl and Hahn-Hägerdal (1997) previously studied about the SmF for lactic acid production from whole wheat using *Lactococcus* (*Lc.*) *lactis* and *Lb. delbrueckii*. According to their results, lactic acid concentrations, yields, and productivities were 106–109 g/l, 0.93–0.95 g/g, and 1.00–1.09 g/l h, respectively. Although we carried out only the SmF using *E. faecalis* RKY1 because it did not have amylolytic activity, it could produce high amount of lactic acid (102 g/l) from whole wheat flour hydrolyzate. Lactic acid productivity and yield were 4.87 g/l h and 0.97 g/g, respectively, which were the highest values among the data presented in Table 5. In SmF using agricultural resources, it is necessary to hydrolyze them into fermentable sugars through enzymatic treatments, as is shown in this paper. This might result in the extra cost for lactic acid production, although the costs for amylolytic enzymes are commercially inexpensive (e.g. 2.95 US$/kg for Termamyl 120L and 3.92 US$/kg for AMG 300L). Åkerberg and Zacchi (2000) previously observed that the major contributor to the operational cost in the hydrolysis step was the cost of the enzyme mixture, which could be reduced by 0.02–0.12 US$/kg using simultaneous saccharification and fermentation.

4. Conclusions

For the economical considerations, lactic acid fermentations from agricultural resources were investigated. Barley, wheat, and corn were found to be good substrates for lactic acid production without any nutrients. In barley flour, 38 g/l of lactic acid could be produced with maximal dry cell weight of 2.3 g/l and lactic acid productivity of 0.88 g/l h. When whole wheat flour was pre-treated by 0.3% (v/v) sulfuric acid treatment and sterilized by filtration, lactic acid productivity and maximal dry cell weight reached to 2.6 g/l h and 5.90 g/l, respectively. By adaptation of *E. faecalis* RKY1 to CSL-based medium, lactic acid productivity and maximal dry cell weight increased to 31% and 12% higher values than those of non-adapted RKY1. The whole wheat flour hydrolyzate could be used for cheap raw material for lactic acid production, and a combination of trace amounts of yeast extract (only 1.5% to saccharified glucose) with CSL resulted in significant improvement of lactic acid productivity and cell growth.

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