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Utilization of by-products derived from bioethanol production process for cost-effective production of lactic acid

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Abstract The by-products of bioethanol production such as thin stillage (TS) and condensed distillers solubles (CDS) were used as a potential nitrogen source for economical production of lactic acid. The effect of those by-products and their concentrations on lactic acid fermentation were investigated using *Lactobacillus paracasei* CHB2121. Approximately, 6.7 g/L of yeast extract at a carbon source to nitrogen source ratio of 15 was required to produce 90 g/L of lactic acid in the medium containing 100 g/L of glucose. Batch fermentation of TS medium resulted in 90 g/L of lactic acid after 48 h, and the medium containing 10 % CDS resulted in 95 g/L of lactic acid after 44 h. Therefore, TS and CDS could be considered as potential alternative fermentation medium for the economical production of lactic acid. Furthermore, lactic acid fermentation was performed using only cassava and CDS for commercial production of lactic acid. The volumetric productivity of lactic acid [2.94 g/(L·h)] was 37 % higher than the productivity obtained from the medium with glucose and CDS.

Keywords Lactic acid · Ethanol process · Condensed distillers solubles · Thin stillage

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Introduction

During the bioethanol production process, aqueous slurry generated after fermentation is passed through a stripper to recover ethanol. The non-volatile components are removed as a product called whole stillage during this step. The whole stillage is usually centrifuged to isolate the fractions of liquid (called as thin stillage, TS) and solid. The remaining TS is then concentrated using multiple evaporators to produce syrup called as condensed distillers solubles (CDS). The CDS can be dried to produce dried distillers grains with solubles (DDGS) and they can be sold as animal feed [7, 10]. An alternative use of CDS should be developed to convert this low-valued CDS into a high-valued product. For example, CDS could be potentially used as a supplement for lactic acid fermentation, because it contains several nutrients that facilitate the growth of microorganisms.

Lactic acid is an important industrial organic acid and a precursor for many industrial compounds. Annual world production of lactic acid is estimated to reach 259,000 metric tons by the year 2012 [12, 16]. There are two optical isomers of lactic acid, L and D; however, industrial application of lactic acid usually requires the L isomer. Biodegradable and biocompatible poly(lactic acid) as a lactic acid polymer has favorable physical properties if L-lactic acid is used as a monomer [22]. The chemical reaction for lactic acid production results in the racemic mixture of L- and D-lactic acid; however, the selected microorganism produces only one lactic acid optical isomer [9]. Therefore, the production of lactic acid via fermentation is of interest for industrial applications as compared to the chemical synthesis of lactic acid.

Although fungi and bacteria are the most widely employed microorganisms for lactic acid production, bacteria have been frequently selected for industrial lactic acid production [9, 16]. Lactic acid bacteria have the ability

to produce lactic acid as a major product of carbohydrate fermentation, and they usually require complex nutrients because of their limited ability to maintain their own growth factors. Bacteria used for the industrial production of lactic acid must have the ability to convert cheap raw materials into lactic acid with minimal nutritional requirements [9]. In addition, cheap raw materials should be effectively utilized for lactic acid production, because lactic acid is a high-volume bulk chemical, but sold at a low price. Raw materials for lactic acid production should be reduced to ensure that the production process is cost-effective, because raw material cost for lactic acid production is ~34 % of the total manufacturing cost [4]. Therefore, it is necessary to develop sugar sources from a variety of bioresources for lactic acid fermentation [18, 23]. However, it is also important to investigate and screen economical fermentation medium.

In this study, the potential use of TS and CDS as a fermentation medium for lactic acid production was evaluated. In addition, lactic acid fermentation was carried out using cassava as a carbon source in a commercial production process.

Materials and methods

Microorganism, media, and culture conditions

Lactic acid-producing bacterium, *Lactobacillus paracasei* subsp. *paracasei* CHB2121 [14], was used in this study. This strain is stocked in the Korean Collection for Type Cultures (Daejeon, Korea) as KCTC11710BP. The medium for cell growth or inoculum preparation consisted of 20 g/L glucose, 5 g/L YE, 4.5 g/L $(NH_4)_2HPO_4$, and 0.012 g/L $MgSO_4 \cdot 7H_2O$. To investigate the effects of YE, TS, and CDS on lactic acid fermentation, glucose concentration was adjusted to 100 g/L. Growth culture and seed culture were performed in 20- and 100-mL vials, respectively. Inoculated vials were incubated at 37 °C on a shaking incubator (FMC-1000, Eyela, Tokyo, Japan) at 200 rpm, and the culture pH was controlled by the manual addition of 10 N NaOH. Fermentor-type fermentations were performed in a 3-L jar fermentor (LiFlus GX, Hanil, Gangneung, Korea) using a 1.5-L working volume. All experiments using the jar fermentor were carried out at 37 °C and 200 rpm, and at pH 6.5. All the fermentations were performed in three replicate bioreactor cultures.

TS and CDS from an ethanol plant

Figure 1 illustrates the processing procedures of dry grind bioethanol and its by-products. The main process is indicated with solid lines and the by-products are indicated by dotted line. The resulting fermented solution was transferred to a distillation tower where ethanol was distilled and then separated into bioethanol and whole stillage. Whole stillage was centrifuged to produce a fraction of liquid and solid. This liquid was referred to TS, and it

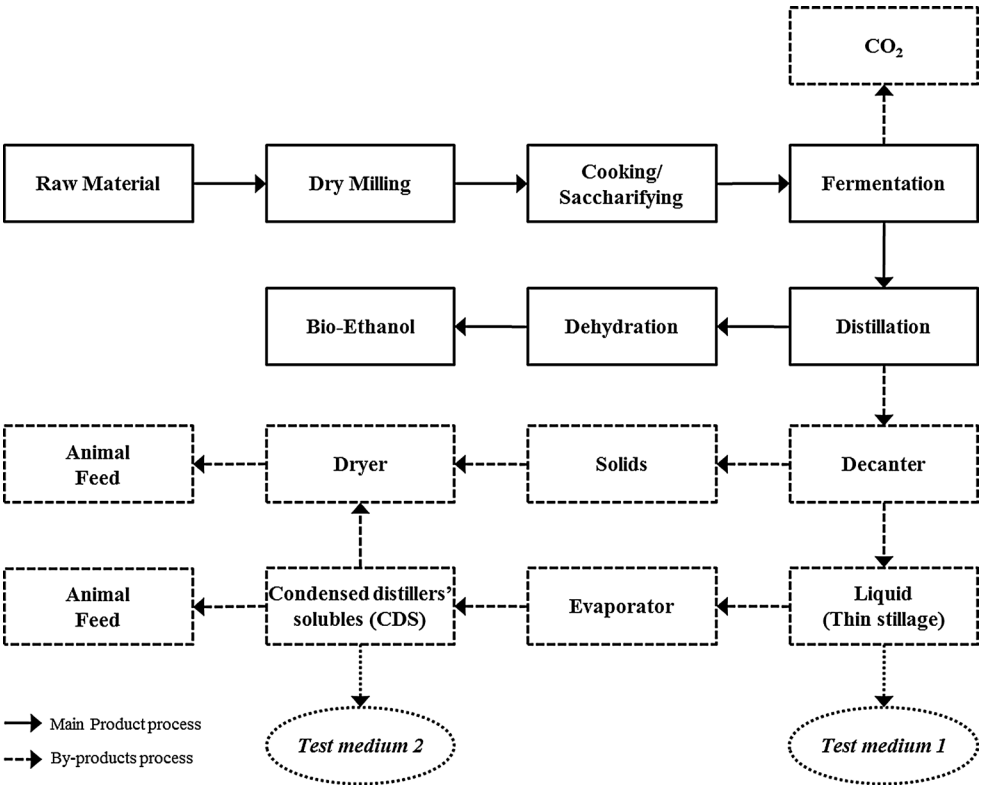

Fig. 1 Schematic illustration of ethanol production process and by-product production process in a commercial ethanol production plant

was concentrated through multiple evaporators to produce CDS. Test mediums 1 and 2 are TS and CDS, respectively, and they were obtained from the commercial ethanol plant, Changhae Ethanol Co., Ltd. (Jeonju, Korea), in which approximately 60 million liters of bioethanol is annually produced using starch feedstocks derived from cassava, rice, wheat, and barley.

Preparation of cassava mash as industrial biomass

Cassava mash was prepared using cassava chips (starch content = approximately 72 % dry basis) imported from Vietnam. Cassava chips were ground using a hammer mill, and passed through a 1-mm screen. The ground cassava powder was provided by Changhae Ethanol Co., Ltd. The glucose of 100 g can be produced from the cassava of 126 g by the enzymatic hydrolysis. For liquefaction, 0.7 g/kg dry matter of commercial α-amylase (Termamyl SC, 120 KNU-S g^{-1}, Novozymes, Bagsvaerd, Denmark) was added, and the mash was heated to 100 °C and liquefied for 90 min. After the liquefying steps were completed, the resulting mash was cooled to a saccharification temperature (50 °C), and Spirizyme Fuel (750 AUG g^{-1}, Novozymes) was added at 0.5 g/kg dry matter. This saccharification step was aseptically performed for 24 h under sterile conditions. The saccharified cassava mash was then used for lactic acid production.

Analytical methods

Cell concentration was quantified using a spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan) at a wavelength of 660 nm, and dry cell weight (DCW) was determined by a calibration curve that correlates the optical density at 660 nm to dry weight (g/L). Glucose and total organic acids, including lactic acid, were quantified using a high-performance liquid chromatography equipped with refractive index detector (Waters 2414; Waters Corp., Milford, MA, USA) and a dual absorbance detector (Waters 2487; Waters Corp.). Samples were applied to an ion-exchange high-performance liquid chromatography column (Aminex HPX-87H; Bio-rad, Hercules, CA, USA) with 4 mM H_2SO_4 as the mobile phase at an elution speed of 0.6 mL/min, and the column temperature was maintained at 65 °C. Total nitrogen and crude protein were analyzed using the Kjeldahl method via the Kjeldahl system (B-339/435/414 system; Buchi, Flawil, Switzerland). Free amino acids were determined using an amino acid analyzer (Waters 2690; Waters Corp.) equipped with a fluorescent detector (Waters 747; Waters Corp.) and an AccQ-TagTM C18 column (3.9 × 150 mm, Waters Corp.). The excitation and emission wavelength used were 250 and 395 nm, respectively.

Results and discussion

Comparison of TS, CDS, and YE

In this study, TS and CDS were used as a fermentation medium for lactic acid production without the addition of other ingredients. To evaluate the nutritional value of TS and CDS as a fermentation medium, the total nitrogen, crude protein, and free amino acid were analyzed and compared with a commercial YE. Among the various complex nitrogen sources, YE is the best choice for both microbial growth and lactic acid production [1, 3]. Furthermore, as Bacto™ yeast extract is one of the most complete and versatile fermentation bionutrients available, it was used as a benchmark comparison.

Thin stillage and CDS contained 10,000 and 85,700 mg/L of total organic acid, which were significantly greater than the levels in the YE (Table 1). YE is produced by the hydrolysis of the yeast after removal of the fermented broth, while TS and CDS are obtained from filtered and concentrated fermented broth. However, these are rather advantageous for the production of lactic acid because most of the produced organic acids are lactic acid (data not shown). Crude protein level in TS and CDS was 5,600 and 84,000 mg/L of the total solution; the nitrogen fractions of TS and CDS are ~16. Table 1 indicates the types and quantities of amino acids present in YE, TS, and CDS. In our previous investigation, the difference in the composition of amino acids depends on the presence or absence of ethanol production in the yeast culture [15]. According to the results of the analysis of free amino acids that play an important role in the growth of microorganisms, 1 L of TS and CDS corresponds to 7.6 and 61.7 g of YE, respectively; this suggests that the use of TS and CDS for lactic acid production would significantly reduce the cost of the fermentation medium in an industrial scale. Moon et al. [15] reported that total free amino acids of YE and ethanol by-products from lactic acid fermentation are comparable, because they are all yeast-derived materials that include amino acids, vitamins, fatty acids, purines, and pyrimidines for their growth and biological activity [13, 19].

Effect of nitrogen source and concentration on lactic acid fermentation

To investigate the effects of nitrogen sources and their concentrations on lactic acid fermentation of the strain CHB2121, several nitrogen sources such as YE, beef extract, corn steep liquor, con steep solid, malt extract, peptone, tryptone, ammonium chloride, ammonium sulfate, and urea were tested. The cell growth and lactic acid production for the CHB2121 strain were excellent when medium containing

Table 1 Comparison of nutritional components of yeast extract, thin stillage, and condensed distillers solubles

Components (mg)	Yeast extract (1 g)	Thin stillage (1 L)	Condensed distillers solubles (1 L)
Total organic acid	72	10,000	85,700
Total nitrogen	140	900	14,000
Crude protein	880	5,600	84,000
Free amino acid	33.4	254.2	2,061.3
Aspartic acid	1.9	6.4	87.6
Glutamic acid	6.2	30.4	130.4
Serine	2.0	9.9	75.8
Glycine	1.1	10.1	70.0
Histidine	2.8	81.3	829.2
Threonine	1.6	5.3	40.8
Arginine	1.3	0.4	17.7
Alanine	3.1	25.4	189.0
Proline	0.9	19.5	137.7
Tyrosine	0.9	7.8	52.7
Valine	2.2	10.5	79.3
Methionine	0.8	4.9	44.7
Isoleucine	2.0	7.8	55.5
Leucine	3.2	15.4	105.0
Lysine	1.5	10.1	83.0
Phenylalanine	2.0	8.9	62.8

only YE was used (data not shown). As with most studies on lactic acid fermentation [2, 3], YE was proved to be the most effective nitrogen source. The influence of YE concentrations on lactic acid fermentation of the strain CHB2121 was investigated using a 3-L fermentor, and batch fermentation was performed using a medium supplemented with 100 g/L of glucose and 2–20 g/L of YE. Figure 2 shows the profiles of dry cell weight, lactic acid production, and glucose consumption in a medium with varied concentrations of YE. Lactic acid production linearly increased as the amount of YE added to the medium increased up to 15 g/L, and then remained constant beyond this value. The highest lactic acid productivity [5.07 g/(L·h)] was obtained when glucose and YE were used in a 6.7:1 ratio. In addition, to produce the lactic acid from 100 g/L glucose by the strain CHB2121, 6.7 g/L YE was required to a minimum, when the glucose–YE ratio is about 15. The CHB2121 strain produced 92 g/L lactic acid from 98 g/L initial glucose and 6.7 g/L YE after 45 h of fermentation. This yielded the lactic acid volumetric productivity, and a maximum dry cell weight of 0.93 g/g, 2.03 g/(L·h), and 6.97 g/L, respectively. The purpose of the study was to compare the effects of various quantities of lactic acid fermentation medium as well as TS and CDS on lactic acid production.

Lactic acid fermentation using TS or CDS

Lactic acid bacteria are generally fastidious microorganisms, and they have complex nutrient requirements owing to their limited ability to biosynthesize B vitamins and amino acids [6]. Therefore, the medium for growth of lactic acid bacteria must be supplemented with a considerable amount of expensive and complex nitrogen sources, such as yeast extract, to produce lactic acid in a reasonable time. Batch fermentation study was performed to evaluate the nutritional availability of TS as an alternative to YE medium for the economical production of lactic acid using the CHB2121 strain. Figure 3 shows the results of the batch fermentations with TS as the nutrient. The CHB2121 strain produced 90 g/L lactic acid from 98 g/L initial glucose during 48 h of fermentation, which resulted in the lactic acid yield, the volumetric productivity, and the maximum DCW of 0.91 g/g, 1.86 g/(L·h), and 5.41 g/L, respectively. Although TS corresponds roughly to YE of 7.9 g/L on the basis of total amino acids, the result of the lactic acid fermentation with TS was poor, as compared to that with YE medium, which consisted of 6.7 g/L YE, 4.5 g/L $(NH_4)_2HPO_4$, and 0.012 g/L $MgSO_4{\cdot}7H_2O$. Kwon et al. [11] reported that when vitamins were added to soybean hydrolysate fermentation medium, similar lactic acid production was obtained as when YE was used. The poor fermentation efficiency was obtained in our experiment using TS probably because the vitamin content of TS is less than that of YE medium. However, this result suggests that, although fermentation efficiency of TS was low compared to YE, it should be enough to use as an inexpensive and alternative fermentation medium to produce lactic acid because TS is significantly cheaper than YE.

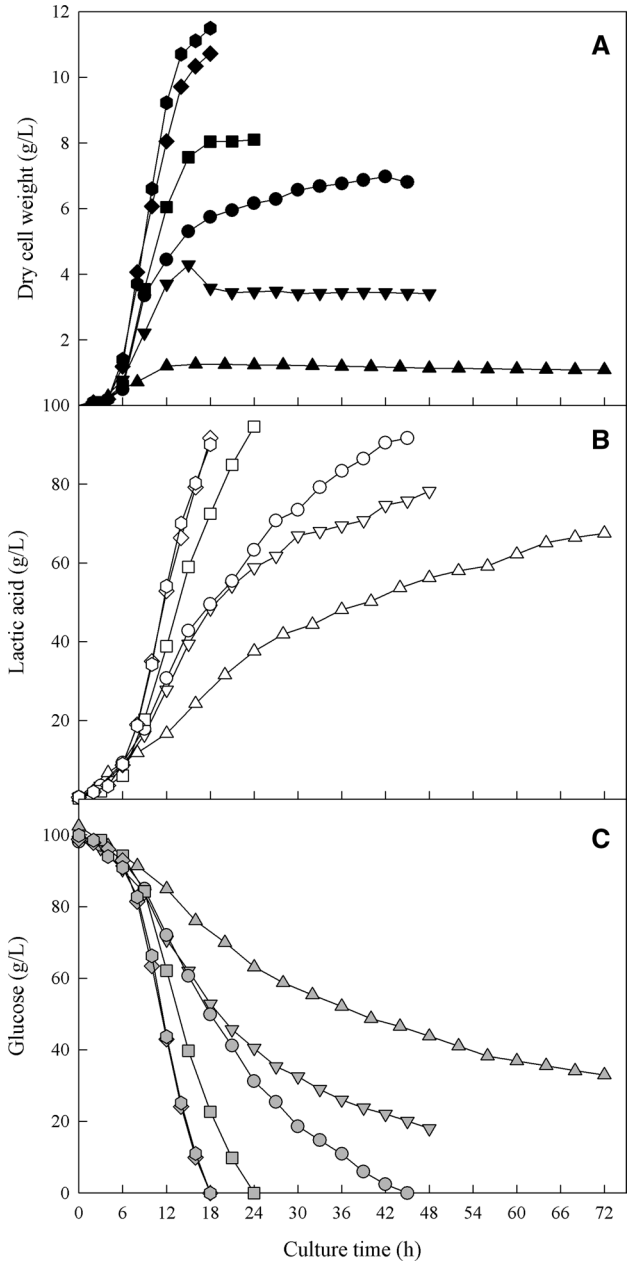

Fig. 2 Lactic acid production, glucose consumption, and cell growth during lactic acid fermentation with different concentrations of YE by *Lactobacillus paracasei* CHB2121. *Filled*, *open*, and *gray-filled symbols* represent DCW, lactic acid, and glucose, respectively. YE concentration (g/L): *triangle* 2, *inverted triangle* 5, *circle* 6.7, *square* 10, *diamond* 15, *hexagon* 20

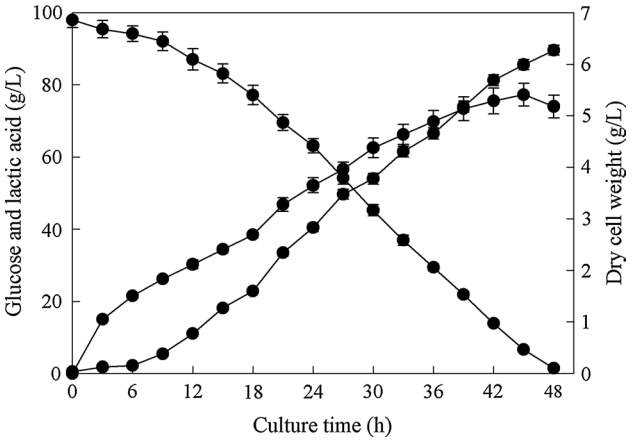

Fig. 3 Profile of lactic acid fermentation by *Lactobacillus paracasei* CHB2121 in TS medium containing 100 g/L glucose. *Circle*, *square*, and *triangle* are glucose, lactic acid, and DCW, respectively

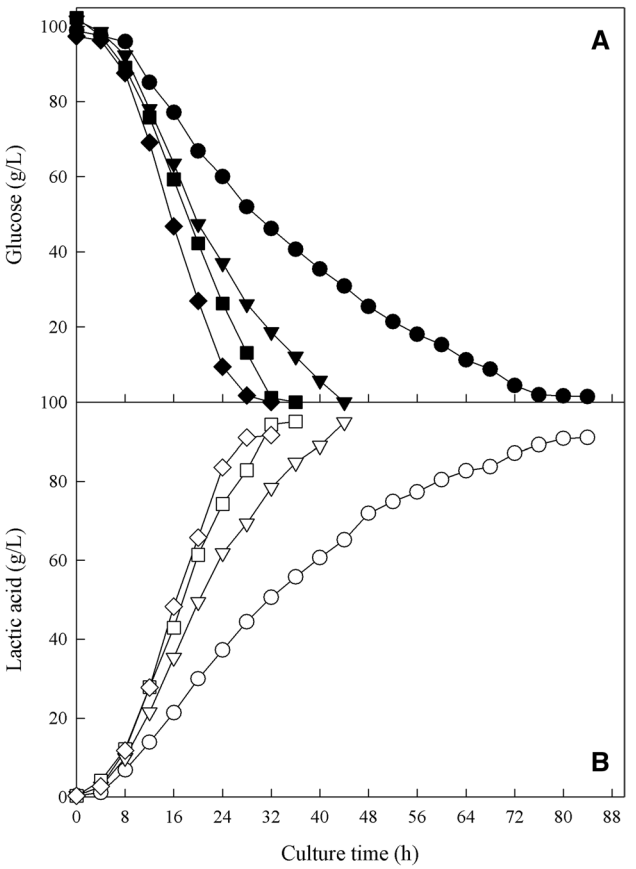

Fig. 4 Effect of CDS contents present in fermentation medium on lactic acid production by batch culture of *Lactobacillus paracasei* CHB2121. *Filled* and *open symbols* are glucose and lactic acid, respectively. CDS contents (%): *circle* 5, *inverted triangle* 10, *square* 15, *diamond* 20

To evaluate the influence of CDS as alternative nutrient source on lactic acid production, 5–20 % CDS was added to 100 g/L glucose. As shown in Fig. 4, the volumetric productivity of lactic acid was improved by increasing CDS. However, there was only a slight improvement (~10 %)

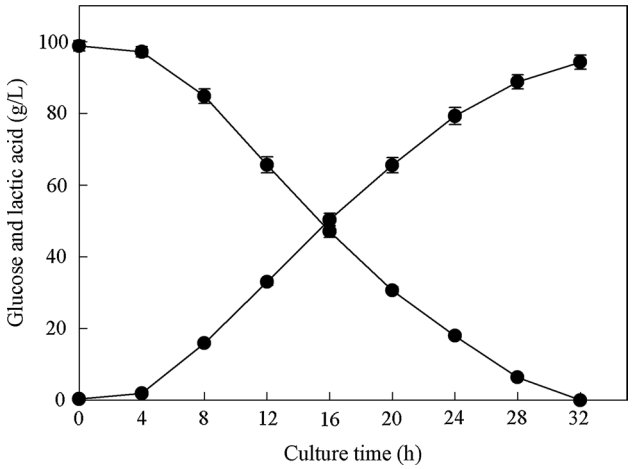

Fig. 5 Profile of lactic acid fermentation by *Lactobacillus paracasei* CHB2121 from saccharified cassava mash with 10 % CDS. *Filled* and *open symbols* are glucose and lactic acid, respectively

in the volumetric productivity of lactic acid. Even though more than 1.5 times higher amounts of amino acids were present in the 15 % CDS medium compared to the 10 % CDS medium, the lactic acid productivity [2.64 g/(L·h)] in the 15 % CDS medium was more than 23 % higher than the 10 % CDS. Considering the effective use of CDS, addition of 10 % CDS for lactic acid fermentation with 100 g/L glucose resulted in enhanced lactic acid production, lactic acid yield, and a volumetric productivity of 95 g/L, 0.93 g/g, and 2.15 g/(L·h), respectively. Moreover, although the total amino acid quantity of 10 % CDS (206 mg/L) was less than that of the YE medium (6.7 g/L), lactic acid fermentation with 10 % CDS was superior to that with YE medium (Figs. 2, 4). To date, recent studies on alternative media for lactic acid fermentation have been conducted using acid treatment, distillation, and enzymatic hydrolysis of yeast [5, 8, 20]. However, these studies resulted in low lactic acid concentration, low volumetric productivity, or low production yield, when compared to the lactic acid fermentation with YE medium. However, according to the results obtained from this study, YE medium could be efficiently replaced with TS and CDS derived from the ethanol production process, which could reduce the production cost of lactic acid. As an additional benefit, our results suggest that the ethanol production process should be integrated with lactic acid production process, which might be significantly important to develop the integrated bioprocess by combination of bioethanol and lactic acid processes (Fig. 5).

Lactic acid fermentation from cassava and CDS for industrial production

Pure sugars and complex nitrogen sources are expensive, whereas lactic acid is a relatively cheap product. According to Tejayadi and Cheryan [21], the cost of raw material is 68 % of total cost for lactic acid production when using whey permeate and YE. Since the raw material cost cannot be reduced by scaling-up the process, cassava and CDS have been considered as attractive nutrient sources. Lactic acid fermentation was carried out using cassava and 10 % CDS without any supplementations. The CHB2121 strain used in this study could produce 94 g/L of lactic acid from 99 g/L glucose present in cassava (Fig. 5). The production yield and the volumetric productivity were 0.95 g/g and 2.94 g/(L·h), respectively. These quantities are far superior to those obtained by fermentation with defined glucose and 10 % CDS, because the nutritional contents in cassava [17] stimulate the growth of microorganism and lactic acid production. Previously, it has been reported that the CHB2121 strain was also able to produce high amount of lactic acid (192 g/L) without substrate or product inhibition [14]. Therefore, fermentation process evaluated in this work using the CHB2121 strain with cassava and bioethanol by-products seems to be appropriate for industrial lactic acid production.

Conclusions

TS and CDS are by-products of the bioethanol process, and they contain essential amino acids and unknown nutrients for growth of lactic acid-producing bacteria. 100,000 KL of bioethanol production-scale processing generates annually 678,000 KL of TS and 33,000 KL of CDS. If TS could be used for lactic acid fermentation, 89.98 g/L lactic acid is fermented to yield 60,700 tons of lactic acid per year without the cost of any added nutrients. For CDS, 95 g/L of lactic acid is fermented using 10 % CDS medium, thus producing 31,300 tons of lactic acid. Although lactic acid productivity is limited to 1.87 g/(L·h) when using TS, approximately twofold higher amount of lactic acid could be produced using TS when compared to the CDS process. In contrast, the lactic acid process using CDS is highly productive, but yields less lactic acid than does the process using TS additives. Taken together, the results couple the ethanol production process to lactic acid fermentation process, which could reduce the total production cost by reduction of added nutrients, water, and wastewater.

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