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Highly efficient production of L-lactic acid from xylose by newly isolated *Bacillus coagulans* C106

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HIGHLIGHTS

- Conversion of xylose to L-lactic acid by a new isolate.
- Fermentation at 50 °C without medium sterilization.
- Lactic acid productivity (7.5 g/L h) reached the highest ever reported.
- Lactic acid titer reached as high as 215.7 g/L.
- Neutralization with $Ca(OH)_2$ improved lactic acid production.

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ABSTRACT

Cost-effective production of optically pure lactic acid from lignocellulose sugars is commercially attractive but challenging. *Bacillus coagulans* C106 was isolated from environment and used to produce L-lactic acid from xylose at 50 °C and pH 6.0 in mineral salts medium containing 1–2% (w/v) of yeast extract without sterilizing the medium before fermentation. In batch fermentation with 85 g/L of xylose, lactic acid titer and productivity reached 83.6 g/L and 7.5 g/L h, respectively. When fed-batch (120 + 80 + 60 g/L) fermentation was applied, they reached 215.7 g/L and 4.0 g/L h, respectively. In both cases, the lactic acid yield and optical purity reached 95% and 99.6%, respectively. The lactic acid titer and productivity on xylose are the highest among those ever reported. $Ca(OH)_2$ was found to be a better neutralizing agent than NaOH in terms of its giving higher lactic acid titer (1.2-fold) and productivity (1.8-fold) under the same conditions.

1. Introduction

Lactic acid has wide applications in food, feed, cosmetics and textile industries as well as in producing poly lactic acid, a promising biodegradable polymer. Currently, lactic acid is commercially produced from glucose derived from starchy materials using *Lactobacillus* species at 30–42 °C (Hofvendahl and Hahn-Hägerdal, 2000; John et al., 2007). The starchy materials not only contribute to a significant proportion of lactic acid production cost but also compete with food supply. To reduce feedstock cost and avoid competition with food supply, the use of cheap, abundant and renewable lignocellulosic biomass as an alternative carbon source has attracted much attention in recent years. Lignocellulose is composed of cellulose (35–50%), hemicellulose (20–40%) and lignin (10–30%) (Saha, 2003). Lactic acid production from lignocellulosic biomass has been extensively reported to use the cellulosic fraction only while leave the hemicelluloses fraction unutilized due to the difficulty in efficient fermentation of pentose sugars such as xylose, which is hard to be metabolized by most microorganisms of commercial interest (Mussatto and Teixeira, 2010). Besides, for lactic acid bacteria that are able to ferment xylose, most of them utilize the phosphoketolase pathway, yielding equal molar amount of acetic acid and lactic acid with a theoretical yield of only 60% (Patel et al., 2006). Due to the co-production of large amount of acetic acid, the cost for lactic acid separation and purification from the fermentation broth is significantly increased, reducing the commercial competitiveness of using hemicellulose sugars as the feedstock.

Some fungi such as *Rhizopus oryzae* have been known to produce L-lactic acid homofermentatively from both glucose and xylose during aerobic growth with lactic acid yields of 71–79%

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on glucose and 54–65% on xylose due to the partial carbon oxidation into CO_2 (Maas et al., 2008). A *Lactobacillus* strain has been genetically engineered for homofermentation of pentoses to lactic acid by redirecting its metabolisms to the pentose phosphate pathway, giving a lactic acid yield of 89%, titer of 41.2 g/L and productivity of 0.69 g/L h (Okano et al., 2009). A newly isolated *Enterococcus mundtii* was reported to produce 94.5 g/L of lactic acid from 104 g/L of xylose with a yield of 85% and a productivity of 1.3 g/L h (Abdel-Rahman et al., 2011). Recently, *Bacillus coagulans* strains have attracted much attention owing to their strong ability to ferment both hexose and pentose sugars to optically pure L-lactic acid using the pentose phosphate pathway with a theoretical yield of 100% (Patel et al., 2006). In addition, *B. coagulans* is moderately thermophilic and capable of fermenting at 50–55 °C, which reduces the risk of microbial contamination as the conventional mesophilic contaminants can hardly grow at this temperature range (Abdel-Banat et al., 2010). Moreover, *B. coagulans* is able to grow in simpler media than MRS medium that is conventionally used for cultivating lactic acid bacteria (Patel et al., 2006). It has been reported that *B. coagulans* fermented xylose to maximally 163 g/L of lactic acid with maximum productivity and yield reaching 1.9 g/L h and 0.93, respectively (Patel et al., 2006; Payot et al., 1999; Ou et al., 2011). In contrast, *Lactobacillus species* converted glucose to as high as 198 g/L of lactic acid with a maximum productivity of 5.6 g/L h (Ge et al., 2011). Therefore, although *B. coagulans* are very commercially attractive for lactic acid production from lignocellulose sugars, their lactic acid titer and productivity still need to be further improved to meet the requirements for commercial production of lactic acid from xylose.

Here we report the lactic acid production from xylose using *B. coagulans* C106, a strain newly isolated from the natural environment of Singapore, which, to the best of our knowledge, gives the highest lactic acid titer and productivity on xylose among those ever reported.

2. Methods

2.1. Isolation of thermophilic lactic acid bacteria

Soil samples were collected from various locations in Singapore. Approximately 2 g of each soil sample was added into 25 mL of enrichment broth (10 g/L of xylose, 5 g/L of yeast extract) and incubated at 50 °C overnight without agitation. The suspensions were serially diluted and plated onto agar plates containing (per liter) 10 g xylose, 5 g yeast extract, 10 g agar and 0.2 g bromocresol green. The plates were then kept in an incubator at 50 °C for 1–2 days until the appearance of colonies with clear color change from green to yellow. The colonies were picked up individually and cultivated in modified mineral salts medium (Patel et al., 2006) containing (per liter) 2 g $(NH_4)_2SO_4$, 2 g KH_2PO_4, 2 g NaCl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.05 g $MnSO_4{\cdot}7H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 10 g yeast extract, 50 g xylose and 30 g $CaCO_3$. After incubation at 50 °C for 48 h without agitation, the strain with the highest L-lactic acid production was selected and designated as C106 for further study.

2.2. Identification of isolated strain C106

The genomic DNA of strain C106 was extracted following the manufacturer's instruction of Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) and used as the template for PCR amplification of 16S rDNA. A partial 16S rDNA region was amplified using the two primers F27 (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR amplification was performed for 30 cycles in an iCycler Thermal Cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). Each cycle consisted of denaturation at 95 °C for 30 s, annealing at 42 °C for 30 s and extension at 72 °C for 1.5 min. The amplified DNA fragments were sequenced at AITbiotech (Singapore) and analyzed by a similarity search in the GenBank database using the BLAST algorithm.

2.3. Batch and fed-batch fermentations

The seed culture of *B. coagulans* C106 was prepared by growing in 100 mL mineral salts medium containing 5% (w/v) xylose and 3% (w/v) $CaCO_3$ in 250 mL conical flasks at 50 °C and 200 rpm for 16 h. Cells were collected by centrifugation at 4000 rpm, 4 °C for 10 min and re-suspended in 50 mL fresh medium of choice. This cell suspension was then used as an inoculum to initiate fermentation.

Batch and fed-batch fermentations of *B. coagulans* C106 were carried out in a 2 L fermenter (Biostat® B plus, Statorius Stedium Biotech, Germany) containing 1 L medium at 50 °C and 100 rpm. The medium was composed of (g/L) $(NH_4)_2SO_4$ 2, KH_2PO_4 2, NaCl 2, $MgSO_4{\cdot}7H_2O$ 0.2, $MnSO_4{\cdot}7H_2O$ 0.05, $FeSO_4{\cdot}7H_2O$ 0.01, yeast extract 10–20 and various amounts of xylose as required. In fed-batch fermentation, xylose powder was supplemented at predetermined time points during the fermentation. Either NaOH (15 M) or $Ca(OH)_2$ (35%, w/v) was automatically added to control the pH at 6.0. All fermentations were conducted without sterilizing the medium prior to fermentation.

2.4. Enzyme assays

During batch fermentations (1 L) of 80 g/L and 150 g/L of xylose with 15 M NaOH as the neutralizing agent, 40 mL of samples were withdrawn for enzyme assays. Cultures were harvested by centrifugation at 4000 rpm at 4 °C for 20 min. Cell pellets were resuspended in 2 mL of extraction buffer (20 mM Tris–HCl, pH 7.5; 10% glycerol, 0.1 mM phenylmethylsulfonyl fluoride and 1 mM dithiothreitol). The resulting cell suspensions were sonicated using a Branson Digital Sonifier® Cell Disruptor (BRANSON ULTRASCHALL, Dietzenbach, Germany) in ice water bath for 5 min (5 s of pulse and 5 s of rest, amplitude 40%). Cell debris was removed by centrifugation (18,000*g*, 4 °C, 30 min). The cell lysates were analyzed for protein concentrations using the Bradford method and subsequently used for enzyme assays.

Lactate dehydrogenase activity was assayed using the Lactate Dehydrogenase Activity Assay Kit (BioVision Inc., Milpitas, CA, USA) following the manufacturer's instructions with minor modification by incubation at 50 °C. One unit of enzyme activity was defined as the amount of enzyme that oxidizes 1 μmol of NADH per min. The activity of D-xylose isomerase was assayed following the method of Dische and Borenfreund (1951). The reaction was conducted in a 1 mL solution containing 70 mM D-xylose, 20 mM $MgCl_2$, 5 mM $MnSO_4$ and 2 mM dithiothreitol in 100 mM Tris buffer (pH 7.5) at 50 °C for 30 min. The D-xylulose formed was quantified using the cysteine–carbazole–sulfuric acid assay by measuring the absorbance at 540 nm. One unit of xylose isomerase activity is expressed as 1 nmol of D-xylulose produced per min.

2.5. Analytical methods

Xylose, lactic acid and acetic acid were analyzed by HPLC (LC-10AT, Shimadzu, Japan) equipped with a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad, Hercules, CA, USA) at 50 °C. Samples were eluted by 12 mM H_2SO_4 at 0.65 mL/min and detected by a refractive index detector. Optical purity of lactic acid was checked using the same HPLC but equipped with a Mitsubishi MCI(R) GEL CRS15W column (50 × 4.6 mm, Mitsubishi Chemical, Japan) at 30 °C using 2 mM $CuSO_4$ as the mobile phase at 0.4 mL/min. The optical purity of L-lactic acid was calculated as follows:

$$\text{Optical purity} = \frac{\text{L-latic acid} - \text{D-latic acid}}{\text{L-latic acid} + \text{D-latic acid}} \times 100\%$$

3. Results and discussion

3.1. Identification of thermophilic lactic acid producer C106

Around 2000 bacterial isolates were obtained from over 400 environmental samples. Among them, strain C106 was found to be the best L-lactic acid producer. Its 16S rDNA sequence (Sq. (A.1) in Appendix) showed a 99.9% identity to those of several *B. coagulans* strains. Therefore, it was identified as belonging to *B. coagulans* and named as *B. coagulans* C106. *B. coagulans* strains have been well known for their strong ability of using both hexose and pentose sugars as the carbon sources (Patel et al., 2006), which is very attractive for producing lactic acid from lignocelluolose, a cheap, abundant and renewable resource. *B. coagulans* C106 is able to produce optically pure L-lactic acid (ee 99.6%) homofermentatively in mineral salts medium under anaerobic conditions with 50 °C as the optimal temperature. The high optical purity of L-lactic acid might be due to the frameshift mutation in the D-lactate dehydrogenase gene of this strain, which introduces a premature stop codon leading to the abnormal shortening of the coded protein. If the missing base is artificially inserted, the resulting amino acid sequence would have very high similarities (93–96%) with those of the D-lactate dehydrogenases from other *B. coagulans* strains (Fig. A1 in Appendix).

3.2. Batch fermentation of xylose

Batch fermentations were conducted at different xylose concentrations using 15 M NaOH as the neutralizing agent (Fig. 1). When xylose concentration was below 117 g/L, *B. coagulans* C106 was able to convert all xylose to L-lactic acid with high yields ($\geqslant$94%), indicating that the pentose phosphate pathway was utilized for lactic acid production (Patel et al., 2006). The lactic acid productivities reached 5.6, 7.5 and 3.7 g/L h at 51, 85 and 117 g/L of xylose, respectively. When the xylose concentration was raised to 151 g/L, the L-lactic acid titer reached only 118.4 g/L and considerable amount of xylose was unconsumed. Obvious decreases in xylose consumption rate were observed when xylose concentration was increased from 85 to 117 g/L and further to 151 g/L (Fig. 1a), possibly indicating a substrate inhibition at higher xylose concentrations (Ou et al., 2011).

During the batch fermentations of 8% and 15% of xylose, cells were harvested at the stationary phase and cell extracts were prepared for measuring xylose isomerase and lactate dehydrogenase activities. In batch fermentation of 8% xylose by *B. coagulans* C106, the specific activity of xylose isomerase reached 1057 U/mg protein, much higher than those (127–464 U/mg protein) reported for the stationary-phase cultures of *Lactobacillus lactis* (Erlandson et al., 2000). The high xylose isomerase activity of *B. coagulans* C106 might be partially responsible for its strong ability of digesting xylose giving the high lactic acid productivities. In batch fermentation of 15% xylose where substrate inhibition was observed, the xylose isomerase activity was 690 U/mg protein, which was only 65% of that at the optimal xylose concentration of 80 g/L. Xylose isomerase is essential for the metabolism of xylose as it converts xylose to xylulose which is then metabolized through either the phosphoketolase pathway or pentose phosphate pathway or both (Tanaka et al., 2002). For instance, after the introduction of the xylose isomerase gene *xylA* from *Escherichia coli*, the recombinant *Corynebacterium glutamicum* was able to utilize xylose as the carbon source with a xylose isomerase activity of ca 0.3 U/mg protein (Kawaguchi et al., 2006).

The lactate dehydrogenase activity in the stationary phase of 8% xylose fermentation was 0.49 U/mg protein, which is comparable to those reported for the *L. lactis* IO-1 cultures (0.44–0.55 U/mg protein) at a xylose concentration of 7% (Tanaka et al., 2002). However, in the fermentation of 15% xylose by *B. coagulans* C106, the specific activity of lactate dehydrogenase was only 0.11 U/mg protein. The lower xylose isomerase and lactate dehydrogenase activities at higher substrate concentrations might be caused by substrate inhibition. It has been proposed that substrate inhibition is mainly caused by the inhibition on the rate of substrate entering into cells, but the detailed mechanism remains unclear (Lin et al., 2004). Mathematical models have been developed to predict the influence of substrate concentration on microbial growth and lactic acid production, suggesting fed-batch fermentation as a good approach to overcome the initial substrate inhibition (Lee et al., 2007).

3.3. Fed-batch fermentation of xylose

Fed-batch fermentations were conducted using 15 M NaOH as the neutralizing agent for pH control (Fig. 2). At 80 g/L of initial xylose with 55 g/L of xylose powder supplemented at 7.5 h (Fig. 2a), the lactic acid productivity (9.1 g/L h) maintained almost

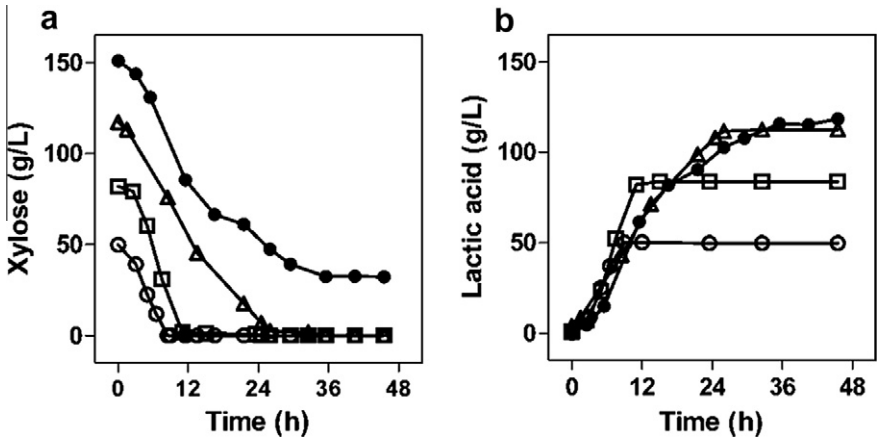

Fig. 1. Time courses of xylose consumption (a) and L-lactic acid production (b) in open batch fermentations with *Bacillus coagulans* C106. Experimental conditions: mineral salts medium, nitrogen source: yeast extract (1% w/v); pH 6.0, neutralizing agent: 15 M NaOH; temperature: 50 °C. Initial xylose concentrations: 51 g/L (○), 85 g/L (□), 117 g/L (△), 151 g/L (●).

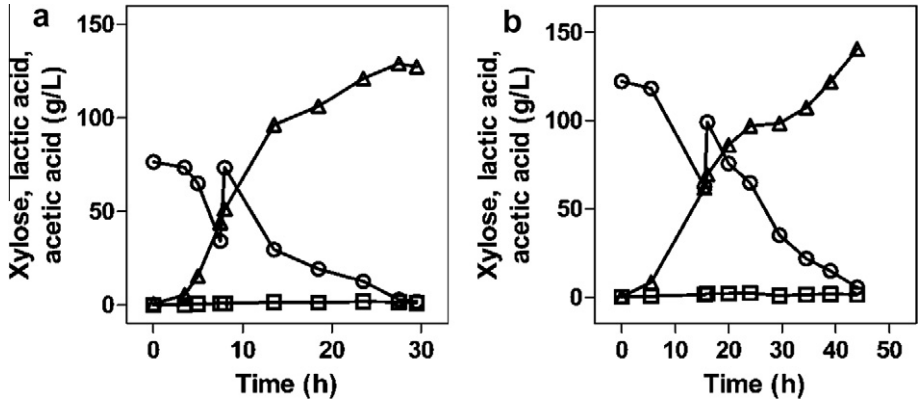

Fig. 2. Time courses of xylose consumption (○), L-lactic acid production (Δ) and acetic acid formation (□) in open fed-batch fermentations with *Bacillus coagulans* C106. Experimental conditions: mineral salts medium, nitrogen source: yeast extract (2% w/v); pH 6.0, neutralizing agent: 15 M NaOH; temperature: 50 °C. (a) Initial xylose concentration: 80 g/L, additional xylose concentration: 55 g/L added at 7.5 h. (b) Initial xylose concentration: 120 g/L, additional xylose concentration: 35 g/L added at 15.5 h.

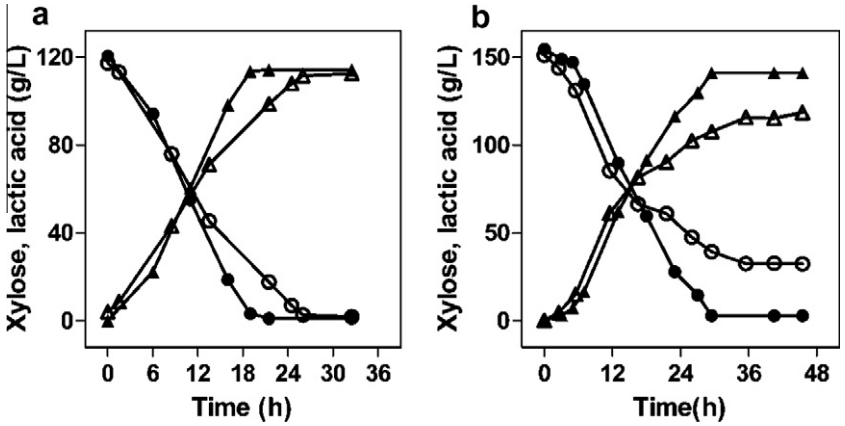

Fig. 3. Time courses of xylose consumption (○, ●) and L-lactic acid production (Δ, ▲) by *Bacillus coagulans* C106 in batch fermentations of 120 g/L (a) or 150 g/L (b) xylose. Experimental conditions: mineral salts medium, nitrogen source: yeast extract (1% w/v); pH 6.0, neutralizing agent: 15 M NaOH (open symbols) or $Ca(OH)_2$ (filled symbols); temperature: 50 °C.

unchanged from 3.5 to 13.5 h. Afterwards, the fermentation rate obviously decreased, which might be due to the inhibition of microbial growth by the high concentration of lactate (Ou et al., 2011). The fermentation was completed within 27.5 h, giving a lactic acid titer of 129.1 g/L, productivity of 4.7 g/L h and yield of 98%. When initiated at 120 g/L of xylose with supplement of 35 g/L of xylose powder at 15.5 h, xylose was completely consumed within 44 h, producing 140.4 g/L of lactic acid with a productivity of 3.2 g/L h and a yield of 94%.

Under similar experimental conditions, fed-batch (120 g/L + 35 g/L) fermentation completely converted all the xylose to lactic acid whereas batch (151 g/L) fermentation failed to ferment almost the same amount of xylose to lactic acid. In addition, the lactic acid productivity and titer were also higher in the fed-batch fermentation (3.2 g/L h and 140.4 g/L, respectively) than in the batch fermentation (2.6 g/L h and 118.2 g/l, respectively). This might be attributed to the reduced substrate inhibition in the fed-batch fermentation.

3.4. Effect of neutralizing agents on fermentation

In both batch and fed-batch fermentations using NaOH as the neutralizing agent, it was observed that the sugar consumption rate started to decline when lactic acid concentration reached 100 g/L (Figs. 1 and 2). This might be attributed to the inhibitions of the growth and fermentation of *B. coagulans* by the accumulated lactic acid, a phenomenon also reported for other bacteria (Goncalves et al., 1997; Iyer and Lee, 1999; Pieterse et al., 2005). The product inhibition of lactic acid bacteria might be caused by (1) collapse of cell membrane due to the change of membrane potential; (2)

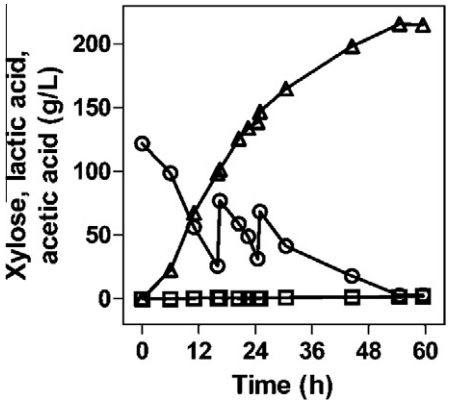

Fig. 4. Time courses of xylose consumption (○), L-lactic acid production (Δ) and acetic acid formation (□) in open fed-batch fermentation with *Bacillus coagulans* C106. Experimental conditions: mineral salts medium, initial xylose concentration: 120 g/L, additional xylose: 80 g/L and 60 g/L added at 16 h and 24.5 h, respectively, nitrogen source: yeast extract (2% w/v); pH 6.0, neutralizing agent: 35% $Ca(OH)_2$; temperature: 50 °C.

Table 1
Comparison of xylose fermentation by *B. coagulans* C106 with those by other bacteria reported in the literature.

Organism	Xylose (g/L)	Lactic acid (g/L)	Productivity (g/L h)	Yield (g/g consumed sugar)	Lactic acid isomer	Temp. (°C)	pH	Neutralizing agent	Nitrogen sources (/L)	Reference
B. coagulans C106	85	83.6	7.5	0.98	L	50	6.0	15 M NaOH	10 g Yeast extract	This work
	80 + 55	129.1	4.7	0.98	L	50	6.0	15 M NaOH	10 g Yeast extract	
	117	112.4	3.7	0.94	L	50	6.0	15 M NaOH	10 g Yeast extract	
	120 + 35	140.4	3.2	0.94	L	50	6.0	15 M NaOH	10 g Yeast extract	
	120	114.2	5.7	0.98	L	50	6.0	35% $Ca(OH)_2$	10 g Yeast extract	
	154	140.9	4.8	0.98	L	50	6.0	35% $Ca(OH)_2$	10 g Yeast extract	
	120 + 60 + 80	215.7	4.0	0.95	L	50	6.0	35% $Ca(OH)_2$	20 g Yeast extract	
B. coagulans 36D1	120	89.1	1.86	0.88	L	50	6.0	7.5% $CaCO_3$, 2 M KOH	10 g Yeast extract	Ou et al. (2011)
	120	102.3	0.71	0.86	L	50	6.0	7.5% $CaCO_3$, 2 M KOH	10 g Peptone, 5 g yeast extract (LB broth)	
	100 + 50 + 50	163.0	0.75	0.87	L	50	6.0	7.5% $CaCO_3$, 2 M KOH	10 g Peptone, 5 g yeast extract (LB broth)	
Enterococcus mundtii QU 25	103.7	94.5	1.31	0.85	L	43	7.0	10 M NaOH	4 g Yeast extract, 8 g meat extract, 10 g peptone (MRS broth)	Abdel-Rahman et al. (2011)
B. coagulans 36D1	30	20.5	0.43	0.80–0.93	L	50	5.0	2 M KOH	5 g Corn steep liquor	Patel et al., 2006
B. coagulans 17C5	30	22.7	0.24		L	50	5.0	2 M KOH	5 g Corn steep liquor	
B. coagulans P4-102B	30	20.5	0.21		L	50	5.0	2 M KOH	5 g Corn steep liquor	
B. coagulans DSM 2314	50	35	0.35	NA[a]	L	54	6.4	20% KOH	10 g Yeast extract	Otto (2006)
Enterococcus casseliflavus IFO 12256	50	39	0.81	0.86	L	30	6.8	4 M NaOH	4 g Yeast extract, 8 g meat extract, 10 g peptone (MRS broth)	Taniguchi et al. (2004)
Lactobacillus vaccinostercus NRIC 1075	50	26	0.93	0.62	L, D	30	6.8	4 M NaOH	4 g Yeast extract, 8 g meat extract, 10 g peptone (MRS broth)	
L. casei subsp. *rhamnous*	80	65	0.27	0.80	L	45	5.7	$CaCO_3$ or NH_4OH	5 g Yeast extract	Iyer et al. (2000)
(ATCC10863)	100	78	0.20	0.78	L	45	5.7	$CaCO_3$ or NH_4OH	5 g Yeast extract	
L. delbrueckii	27	25.1	0.13	0.93	NA[a]	45	5.0	6 M NH_4OH	30 g Yeast extract	Thomas (2000)
	25	22.5	0.28	0.90	NA[a]	45	5.0	6 M NH_4OH	27.5 g Tryptone	
L. pentosus	23	12.9	0.27	0.56	NA[a]	33	6.5	6 M NH_4OH	27.5 g Tryptone	
L. lactis IO-1	70.3	33.3	0.67	0.67	L	37	6.0	3 M NaOH	5 g Yeast extract, 10 g polypeptone	Tanaka et al. (2002)
	69.9	20.6	NA[a]	0.55	L	37	6.0	3 M NaOH	5 g Yeast extract, 10 g polypeptone	
L. lactis IO-1	51.2	24	0.6	0.47	L	37	6.0	3 M NaOH	5 g Yeast extract, 5 g polypeptone	Ishizaki et al. (1992)
L. xylosus	31	13	0.24	0.48	L	30	6.4–6.5	4 M NaOH	7 g Yeast extract, 3 g peptone	Tyree et al. (1990)

[a] NA, not available.

acidification of cytosol; (3) anions accumulation inside the cells; (4) presence of high concentration of solutes (Axe and Bailey, 1995; Hartke et al., 1996; Nakano et al., 2012).

Trapping lactic acid as calcium lactate during the fermentation of *Lactobacillus delbrueckii* has been shown to be an effective means to improve lactic acid production (Nakano et al., 2012). Therefore, $Ca(OH)_2$ (35%, w/v) was tested as the neutralizing agent to control the pH during the fermentations of *B. coagulans* C106 and the results were compared with those obtained using NaOH as the neutralizing agent.

As shown in Fig. 3, in batch fermentations of 120 g/L and 154 g/L of xylose with $Ca(OH)_2$ as the neutralizing agent, the lactic acid productivities reached 5.7 g/L h and 4.8 g/L h, respectively, which are 1.5-fold and 1.8-fold of those obtained using NaOH as the neutralizing agent. In addition, 154 g/L of xylose was completely consumed using $Ca(OH)_2$ as the neutralizing agent while considerable amount of xylose was left unutilized when NaOH was used, resulting in a higher lactic acid titer (140.9 g/L vs 118.4 g/L) (Fig. 3b).

Similarly, fed-batch fermentation was applied to further improve the lactic acid production using $Ca(OH)_2$ as the neutralizing agent. Fermentation was initiated from 120 g/L of xylose with supplements of 80 g/L and 60 g/L of xylose powder at 16 h and 24.5 h, respectively, when the xylose concentration was decreased to 30 g/L (Fig. 4). The fermentation rate maintained almost unchanged until 25 h when lactic acid concentration reached 147 g/L and began to slow down afterwards. The lactic acid titer, productivity and yield reached 216 g/L, 4.0 g/L h and 95%, respectively, after 54.5 h when xylose was completely consumed.

Therefore, $Ca(OH)_2$ is a more suitable neutralizing agent than NaOH in terms of getting higher lactic acid titer and productivity under the same conditions. This might be attributed to the reduced product inhibition owing to the formation of calcium lactate rather than sodium lactate. High concentration of sodium salts have been reported to inhibit the growth and lactic acid production of *B. coagulans* (Walton et al., 2010). The lower solubility of calcium lactate might be the major reason for the higher lactic acid titer and productivity. At high lactate concentrations, a large portion of calcium lactate was precipitated, reducing the level of soluble lactate thus alleviating the lactate inhibition. For instance, the solubility of calcium lactate at 50 °C is 128.1 g/L (Cao et al., 2001). Theoretically, when calcium lactate is produced at amounts higher than the solubility, it would be precipitated. Therefore, the inhibition by lactate would be maintained at this level regardless of the amount of total lactate produced. In contrast, sodium lactate would exist in a soluble form at all concentration levels as it is miscible with water. Besides, the lactate molarity might also affect the growth

and productivity of lactic acid bacteria such as *L. delbrueckii* (Nakano et al., 2012). The molarity of calcium lactate is only half of that of sodium lactate at the same lactate concentration. A lower lactate molarity is presumed to be favorable for reducing the product inhibition (Nakano et al., 2012).

3.5. Comparison of xylose fermentation by various bacteria

Table 1 lists the comparison of xylose fermentation by *B. coagulans* C106 with those by other bacteria reported in the literature. It is clear that *B. coagulans* C106 gives the highest lactic acid titer, productivity and yield. For example, *B. coagulans* C106 gave a lactic acid productivity as high as 7.5 g/L h, which is four times higher than the highest productivity reported for *B. coagulans* 36D1 (1.9 g/L h). It is worth mentioning that *B. coagulans* C106 is able to grow and ferment in simple mineral salts medium containing only 1–2% of yeast extract as the organic carbon source, which is advantageous over the conventional lactic acid bacteria for cost-effective production of lactic acid. The conventional lactic acid bacteria such as *Lactobacillus* and *Enterococcus* require a medium rich in organic nitrogen sources for their growth and fermentation. The high lactic acid titers and productivities achieved by *B. coagulans* C106 in xylose fermentation are comparable to those recently reported for glucose fermentation by *Bacillus* sp. WL-S20 and *Bacillus subtilis* MUR1. In fed-batch fermentation of glucose by *Bacillus* sp. WL-S20, 180–225 g/L of L-lactic acid was produced within 112–216 h at yields of 98–99% (Meng et al., 2012), while a *Bacillus subtilis* mutant MUR1 was reported to produce 99–183 g/L of L-lactic acid within 12–52 h at a yield of 98.5% (Gao et al., 2012).

4. Conclusions

A newly isolated *B. coagulans* C106 was used for converting xylose to optically pure L-lactic acid at 50 °C in simple medium without requirement of sterilization. High lactic acid yield, titer and productivity were achieved in both batch and fed-batch fermentations, which are the highest among those ever reported for lactic acid production from xylose. $Ca(OH)_2$ was found to be a better neutralizing agent than NaOH in terms of getting higher lactic acid titer and productivity under the same conditions. The excellent performances of *B. coagulans* C106 makes it a very promising strain for industrial production of L-lactic acid from lignocellulose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.01.011.

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