Ann Microbiol (2013) 63:39–44
DOI 10.1007/s13213-012-0441-8

ORIGINAL ARTICLE

Production of succinic acid and lactic acid by *Corynebacterium crenatum* under anaerobic conditions

Xiaoju Chen · Shaotong Jiang · Xingjiang Li · Lijun Pan · Zhi Zheng · Shuizhong Luo

Received: 18 October 2011 / Accepted: 23 February 2012 / Published online: 19 April 2012

Abstract A new succinic acid and lactic acid production bioprocess by *Corynebacterium crenatum* was investigated in mineral medium under anaerobic conditions. *Corynebacterium crenatum* cells with sustained acid production ability and high acid volumetric productivity harvested from the glutamic acid fermentation broth were used to produce succinic acid and lactic acid. Compared with the first cycle, succinic acid production in the third cycle increased 120% and reached 43.4 g/L in 10 h during cell-recycling repeated fermentations. The volumetric productivities of succinic acid and lactic acid could maintain above 4.2 g/(L·h) and 3.1 g/(L·h), respectively, for at least 100 h. Moreover, wheat bran hydrolysates could be used for succinic acid and lactic acid production by the recycled *C. crenatum* cells. The final succinic acid concentration reached 43.6 g/L with a volumetric productivity of 4.36 g/(L·h); at the same time, 32 g/L lactic acid was produced.

X. Chen · S. Jiang (✉) · X. Li · L. Pan · Z. Zheng · S. Luo
School of Biotechnology and Food Engineering,
The Key Laboratory for Agricultural Products Processing
of Anhui Province, Hefei University of Technology,
No.193 Tunxi Road,
Hefei City 230009 Anhui Province, China
e-mail: jiangshaotong@yahoo.com.cn

X. Chen
e-mail: chenxiaoju2012@hotmail.com

X. Li
e-mail: lixingjiang1978@163.com.cn

L. Pan
e-mail: pljhfut@tom.com

Z. Zheng
e-mail: zhengzhi1971@sina.com.cn

S. Luo
e-mail: luoshuizhong@163.com.cn

Keywords *Corynebacterium crenatum* · Succinic acid · Lactic acid · Wheat bran hydrolysate · Anaerobic fermentation

Introduction

Biomass hydrolysate has high potential as a renewable resource for the future. Production of chemicals, including organic acids and ethanol, by biological fermentation processes from biomass-derived sugars has attracted great interest (Nigam 2001; Zhang et al. 2007; Zheng et al. 2009). Wheat bran is the main by-product of the flour industry. More than 20 million tons of wheat bran is produced every year in China. At present, most wheat bran is used as forage without any further processing and the price is low. Finding an effective use for wheat bran has great significance to the wheat processing industry.

Lactic acid and succinic acid are valuable due to their long history of use in the food, chemical and pharmaceutical industries (Sauer et al. 2008). Because of the potential usage in biodegradable plastics, lactic acid and succinic acid has been attracted a lot of attention (John et al. 2007; Zwicker et al. 1997). Production of lactic acid and succinic acid by microbial fermentation using renewable carbohydrates has been reported (Song and Lee 2006; Zeikus et al. 1999). Although many researchers have tried to produce lactic acid and succinic acid from renewable carbohydrates, there are still some problems, such as detoxification of biomass hydrolysates, low volumetric productivity and complex nutrients. Lactic acid bacteria (LAB) can produce lactic acid with high productivity and high yield, but complex nutrients such as whey and yeast extracts are needed (Litchfield 1996; Zhou et al. 2003). Although the succinic acid concentration produced by *Actinobacillus succinogenes* and genetically

modified *Escherichia coli* can reach 100–110 g/L, the low productivity is insufficient for industrial applications (Guettler et al. 1996; Vemuri et al. 2002).

Millions of tons of amino acids are produced every year in China. Along with the process, the large amount of bacterial biomass generated is usually used as animal forage. *Corynebacterium crenatum* is one of the main amino acid production microorganisms, and has been used for many years in the practical production of amino acids such as glutamic acid and arginine (Cao et al. 2009; Xu et al. 2009). It is known that a small amount of lactic acid and succinic acid are produced during the production of glutamic acid when the dissolved oxygen in the medium is insufficient. When culture conditions become anaerobic, lactic acid and succinic acid are produced as the main products of *C. crenatum*. The cells can be packed at high cell density to achieve high volumetric productivity after glutamic acid fermentation is finished. There are two main advantages. First, it can make full use of the cells from glutamic acid fermentation broth. Second, it can solve the problem of low succinic acid productivity.

In this report, we first investigated the feasibility of using *C. crenatum* harvested from glutamic acid fermentation broth at the end of the glutamic acid fermentation to produce lactic acid and succinic acid in mineral medium under anaerobic conditions. We focused on organic acid volumetric productivity and sustained acid production ability in order to demonstrate the practical application of the bioprocess.

Materials and methods

Microorganism

Corynebacterium crenatum CICC 20219 was cultured and stored in our own laboratory (Key Laboratory for Agricultural Products Processing of Anhui Province, Hefei University of Technology, Hefei, China)

Culture medium

The preculture medium contained (per liter): glucose 25 g; urea 2.5 g; corn steep liquor 20 g; KH_2PO_4 1 g; $MgSO_4{\cdot}7H_2O$ 0.4 g, sterilized at 115°C for 15 min.

The glutamic acid production medium (nutrient medium) contained (per liter): glucose 80 g; $(NH_4)_2SO_4$ 20 g; corn steep liquor 3 g; KH_2PO_4 1 g; $MgSO_4{\cdot}7H_2O$ 0.4 g; $FeSO_4{\cdot}7H_2O$ 10 mg; $MnSO_4{\cdot}H_2O$ 10 mg; biotin 0.2 mg; thiamine 0.2 mg; $CaCO_3$ 50 g, sterilized at 115°C for 15 min. Glucose and $CaCO_3$ were autoclaved separately and added to the glutamic acid production medium.

The organic acid production medium (mineral medium) contained (per liter): KH_2PO_4 0.5 g; K_2HPO_4 0.5 g; $MgSO_4{\cdot}7H_2O$ 0.5 g; $FeSO_4{\cdot}7H_2O$ 6 mg; $MnSO_4{\cdot}H_2O$ 4.2 mg; biotin 0.2 mg and 0.2 mg thiamine (Okino et al. 2005), sterilized at 115°C for 15 min, glucose and $MgCO_3$ were autoclaved separately and added to the organic acid production medium.

Experimental setup

Corynebacterium crenatum was first grown in preculture medium and incubated in a rotary shaker at 200 rpm, 30°C for 12 h. A 7-L fermentor with an initial glutamic acid production medium volume of 5 L was inoculated 10% of preculture and cultured at 30°C for glutamic acid production with agitation of 300 rpm and aeration of 5 L/(L·min). The pH was maintained at 7.0 by supplying sterilized $CaCO_3$. When glutamic acid production was finished, *C. crenatum* cells were harvested from the glutamic acid fermentation broth by centrifugation (5,000 rpm, 4°C, and 10 min), and then the cells were inoculated into the organic acid production medium to produce organic acid. The organic acid production medium was aerated with CO_2 gas for 10 min to remove oxygen before the inoculation. The organic acid fermentation process was conducted in a 3-L fermentor containing 1.5 L organic acid production medium and incubated at 30°C with no aeration. The agitation speed was 100 rpm. During organic acid fermentation, the pH was maintained at 7.0 by supplying with sterilized $MgCO_3$. All experiments were repeated three times.

Wheat bran hydrolysis method

Wheat bran (100 g) was transferred into a 2-L stirred vessel equipped with a temperature controller. The vessel contained 400 mL 1.5% H_2SO_4. Hydrolysis was performed at 90°C for 20 h (Li et al. 2010). The suspension was then centrifuged at 8,000 rpm for 15 min. The supernatants were used as carbon source for production of acid.

Analytical methods

Fermentation samples were centrifuged (10,000 rpm, 4°C, 10 min), and the supernatants were chilled for sugar- and organic acids-analysis. Organic acids were extracted from fermented broth using 0.5 mol/L H_2SO_4. Organic acids were quantified by high-performance liquid chromatography equipped with ultraviolet detector (UV 2996, Waters, Milford, MA) using a Purospher STAR C18250×4.6 (5 μm) (Merck, Whitehouse Station, NJ) column. The column was used at 30°C and H_2SO_4 (5 mmol/L) was used as mobile phase at a flow rate of 0.8 mL/min. Sugar concentration was determined by high-performance liquid

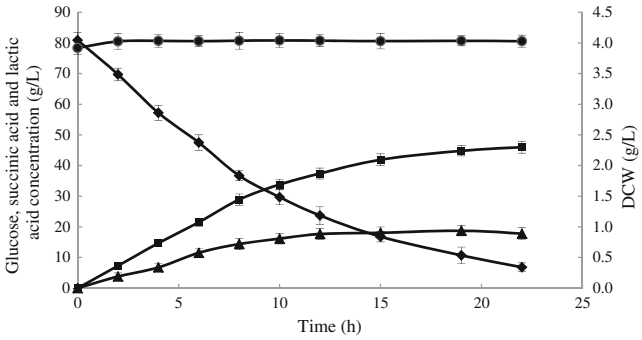

Fig. 1 Production of organic acid by *Corynebacterium crenatum* under anaerobic conditions: *filled circles* dry cell weight (DCW), *filled diamonds* glucose, *filled squares* lactic acid, *filled triangles* succinic acid

chromatography with a scattering detector (ELSD 2424, Waters, Milford, MA). A carbohydrate analysis (3.9× 300 mm, Waters, Dublin, Ireland) column was used. The column was maintained at 30°C and acetonitrile (80%) was used as mobile phase at a flow rate of 1 mL/min.

Cell growth was monitored at 610 nm using a spectrophotometer (TU-1901, PERSEE, Beijing, China). Dry cell weight (DCW) was obtained according to the method of Okino et al. (2008). DCW can be calculated by the value of OD_{610nm} according to the formula: OD_{610nm} 1=0.34 g/L DCW. Protein contents were determined by the method of Bradford (1976).

Enzyme assays

To analyze intracellular enzyme activities, cells were harvested by centrifugation (10,000 rpm, 4°C, 10 min), and washed twice with chilled solution of Tris–HCl (50 mmol/L, pH 7.5) contained 15% glycerol. Washed cells were suspended in the same buffer and sonicated on ice for 90 cycles, with a working time of 1 s and interval of 5 s at the power of 200 W using an ultrasonic disruptor (GA92-IID, ShangJia Biotechnology, WuXi, China). Cell debris was removed by centrifugation (12,000 rpm, 4°C, 20 min), and the supernatant was used for measurement of enzyme activity.

The activity of lactic dehydrogenase (LDH) was assayed according to the method of Bunch et al. (1997). the activities of malic dehydrogenase (MDH) and phosphoenolpyruvate carboxylase (PEPC) were assayed according to the method of Wu et al. (2007). Glutamate dehydrogenase (GDH) activity was assayed according to the method of Bormann et al. (1992).

Results and discussion

Production of succinic acid and lactic acid by *C. crenatum* under anaerobic conditions

When glutamic acid production was finished, the cells were harvested to study the features of organic acid production. The harvested cells were inoculated into a 3-L fermentor containing 1.5 L organic acid production medium. As Fig. 1 shows, the proliferation of *C. crenatum* was arrested and glucose could be consumed by *C. crenatum* under anaerobic conditions. Compared with the metabolites produced under aerobic conditions, succinic acid and lactic acid were the main products under anaerobic conditions. About 19.8 g/L succinic acid and 46 g/L lactic acid were produced from 74 g/L glucose within 22 h. The yield of succinic acid and lactic acid was 0.27 g/g and 0.62 g/g, respectively (g/g, grams acid formed/grams glucose utilized) (Table 1).

Compared with aerobic conditions, more succinic acid and lactic acid were produced under anaerobic conditions. The metabolic flux in *C. crenatum* was redistributed when changing from aerobic to anaerobic conditions. The metabolic flux to lactic acid and succinic acid increased significantly; meanwhile, flux to glutamic acid decreased. The intracellular LDH, MDH and GDH activities were analyzed (Table 2). Under anaerobic conditions, the activities of LDH and MDH were both higher than the value detected under aerobic conditions, which increased by 6.4-fold and 3-fold respectively. Meanwhile, GDH enzyme activity decreased from 0.83 to 0.09 U/mg protein due to the reaction from isocitric acid to α-ketoglutarate being suppressed under

Table 1 Metabolites produced under different aeration condition by *Corynebacterium crenatum*

Aeration condition	Medium	Substrate consumed (g/L)	Glutamic acid (g/L)	Succinic acid (g/L)	Lactic acid (g/L)
Aerobic[a]	Nutrient[c]	79.00±0.35[e]	21.60±0.42	0.40±0.03	1.90±0.21
Anaerobic[b]	Mineral[d]	74.00±0.40	0.80±0.08	19.80±0.33	46.00±0.45

[a] Aerobic conditions were used for production of glutamic acid

[b] Anaerobic conditions were used for production of organic acid

[c] Nutrient medium for glutamic acid production as described in Materials and methods

[d] Mineral medium for organic acid production as described in Materials and methods

[e] The value given is mean ± standard deviation from three parallel replicates

Table 2 Specific enzyme activities under different aeration conditions. Values given are mean ± standard deviation from three parallel replicates. *MDH* Malic dehydrogenase, *LDH* lactic dehydrogenase, *GDH* glutamate dehydrogenase

	Specific activity (U/mg protein)		
Aeration condition	MDH	LDH	GDH
Aerobic[a]	0.70±0.02	0.25±0.03	0.83±0.04
Anaerobic[b]	2.10±0.03	1.60±0.05	0.09±0.01

[a] Aerobic conditions for production of glutamic acid

[b] Anaerobic conditions for production of organic acid

anaerobic conditions. Also, the TCA cycle could not continue. One mole of glucose can generate two moles of NADH through the EMP pathway, and NADH accumulation can suppress the glycolytic pathway with glyceraldehyde 3-phosphate dehydrogenase inhibition (Danshina et al. 2001; Dominguez et al. 1993). In order to regenerate NAD^+ to continue glycolysis, the production pathways of succinic acid and lactic acid are activated. Synthesizing succinic acid and lactic acid can utilize NADH and allow NAD^+ to regenerate. These changes in intracellular enzymes activities could account for the different metabolites produced by *C. crenatum* when cultured under different conditions.

Effect of propane diacid and fluoroacetic acid on production of succinic acid by *C. crenatum*

Succinic acid is produced using the reductive arm of the TCA cycle by *E. coli* and *A. succinogenes* (Lu et al. 2009; McKinlay and Vieille 2008). Propane diacid cannot be catalyzed to dehydrogenize by fumarate reductase, so propane diacid can be used as an inhibitor of fumarate reductase. If succinic acid is produced by the reductive arm of the TCA cycle, the addition of propane diacid can decrease production of succinic acid, while the addition of fluoroacetic acid can be used to prove the role of glyoxylate cycle in the production of succinic acid, because fluoroacetic acid can prevent the isomerization of citric acid and restrain the formation of isocitric acid (Wang et al. 2002). In order to demonstrate whether *C. crenatum* also utilizes the reductive arm of the TCA cycle as the main pathway for succinic acid formation, experiments were designed to investigate the effect of propane diacid and fluoroacetic acid on the production of succinic acid.

As shown in Table 3, the addition of propane diacid had a significant influence on the production of succinic acid, while fluoroacetic acid had no obvious effect. The final concentration of succinic acid decreased along with the increasing addition of propane diacid. At a propane diacid concentration of 2 g/L, only 10.6 g/L succinic acid was obtained. The succinic acid concentration decreased 44% compared with the control, indicating that most of the succinic acid was produced from fumarate and implying that the reductive arm of the TCA cycle is the main pathway for succinic acid formation in *C. crenatum*. Moreover, production of lactic acid was also influenced slightly by the addition of propane diacid. Less succinic acid produced might possibly lead to an imbalance between NADH and NAD^+ levels, equivalent to suppression of the glycolytic pathway with glyceraldehyde 3-phosphate dehydrogenase inhibition resulting from NADH accumulation (Danshina et al. 2001; Dominguez et al. 1993).

Sustained organic acid production in high-cell density

In an efficient industrial bioprocess, the organic acid production rate should ideally stay relatively constant with increasing cell density to sustain the production rate for as long as possible. The relationship between cell concentration and the production rates of organic acids was investigated. For long-term stability of organic acid production, cells need to be recycled and inoculated into fresh organic acid production medium repeatedly.

As shown in Fig. 2, an approximately linear relationship exists between cell concentration and the production rates of lactic acid and succinic acid. When dry cell concentration was 60 g/L, 59 g/L lactic acid and 23 g/L succinic acid were produced within 2 h. The maximum production rate of lactic acid and succinic acid was 29.5 g/(L·h) and 11.5 g/(L·h), respectively. In repeated cycle fermentations, the succinic acid concentration in the first cycle reached 19.8 g/L, while 54.5 g/L lactic acid was also produced. Surprisedly, the succinic acid concentration in the third cycle increased

Table 3 Effect of propane diacid and fluoroacetic acid on production of organic acid by *C. crenatum*. Value given are the mean ± standard deviation from three parallel replicates

Product	Control[a]	Propane diacid (g/L)		Fluoroacetic acid (g/L)	
		0.5	2	0.1	1
Succinic acid	18.70±0.30	13.80±0.42	10.60±0.23	18.50±0.41	18.00±0.53
Lactic acid	52.00±0.36	50.50±0.76	49.20±0.38	52.40±0.64	51.80±0.82

[a] No addition of propane diacid or fluoroacetic acid

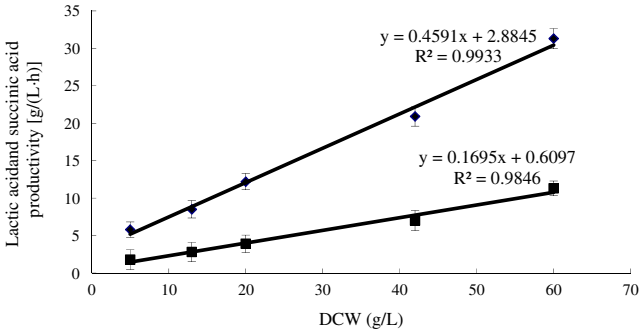

Fig. 2 Relationship between cell concentration and organic acid productivity: *filled diamonds* lactic acid, *filled squares* succinic acid

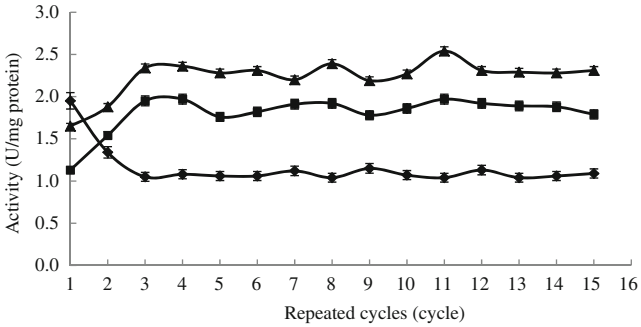

Fig. 4 Specific activities of phosphoenolpyruvate carboxylase (PEPC; *filled squares*), lactic dehydrogenase (LDH; *filled diamonds*) and malic dehydrogenase (MDH; *filled triangles*) in repeated fermentations

significantly compared with cycle 1 and reached 43.4 g/L, and the concentration of lactic acid decreased to 30.3 g/L. The succinic acid and lactic acid concentration was then maintained at 40–43 g/L and 30–33 g/L, respectively, in 10 repeated cycles (from cycle 3 to 12). Glucose was consumed completely in 10 h for each cycle, and all the above fermentations were with an initial glucose concentration of 80 g/L. A total of 780 g glucose was consumed, and 423 g succinic acid and 310 g lactic acid were produced in 100 h. The average volumetric productivities of succinic acid and lactic acid were 4.2 g/(L·h) and 3.1 g/(L·h), respectively. The data demonstrate that *C. crenatum* can produce organic acid with high productivity for a time period of at least 100 h.

Though the productivity of lactic acid by *C. crenatum* was not the highest, the succinic acid volumetric productivity [4.2 g/(L·h)] by *C. crenatum* was higher than most other microorganisms reported by Song and Lee (2006), except for genetically modified *Corynebacterium glutamicum* [11.8 g/(L·h); the strain used was a mutant with *ldhA* gene knockout and pyruvate carboxylase gene overexpression] and *Anaerobiospirillum succiniproducens* [10.4 g/(L·h), the study was carried on a membrane-reactor-electrodialysis system; Meynial-Salles et al. 2008; Okino et al. 2008].

From cycle 1 to 3, succinic acid concentration increased 120%, while lactic acid concentration decreased 45%. The reason for this was that, with longer culture time under anaerobic conditions, the reductive arm of the TCA cycle was activated thoroughly. Enzymes activities were analyzed to support this supposition. In Fig. 3, it can be seen that the enzyme activity of MDH and PEPC increased in the first two cycles. The reductive arm of the TCA cycle had then been activated thoroughly and no significant changes in LDH, MDH and PEPC activities were observed in the last 13 repeated fermentation cycles (Fig. 4). Due to the enhancement of PEPC activity, the metabolic flux at the phosphoenolpyruvate (PEP) node was redistributed. Consequently, more metabolic flux was redistributed to the C_4 pathway and more succinic acid was produced.

Organic acid production from wheat bran hydrolysate

Before fermentation, the wheat bran hydrolysate was concentrated and the initial glucose concentration in organic acid production medium was 80 g/L. The cells for inoculation had been used for two cycles as described above. The final succinic acid concentration reached 43.6 g/L with a volumetric productivity of 4.36 g/(L·h) (Fig. 5). At the same

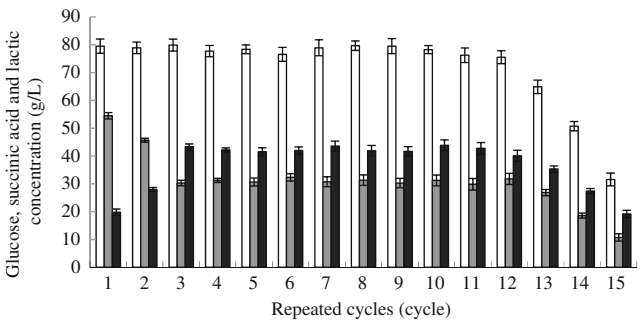

Fig. 3 Stability of organic acid production in repeated fermentations by *C. crenatum*: *white bars* consumed glucose, *gray bars* lactic acid, *black bars* succinic acid

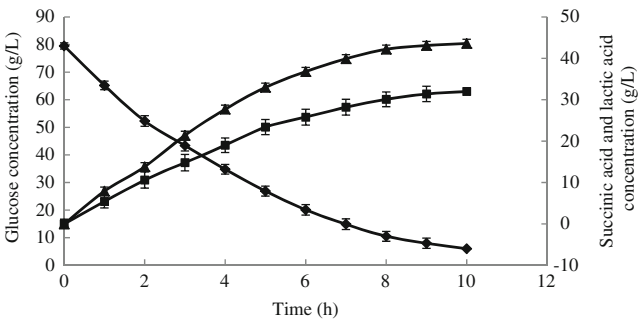

Fig. 5 Organic acid production from wheat bran hydrolysate by *C. crenatum*: *filled diamonds* glucose, *filled squares* lactic acid, *filled triangles* succinic acid

time, 32 g/L lactic acid was produced. The total acid yield was 1.03 g/g, which was higher than the fermentations using analytical reagent grade glucose as substrate. The reason for this was that there are factors hydrolyzed from the wheat bran that are beneficial for organic acid production. The results demonstrate that wheat bran hydrolysates can be used for organic acid production under anaerobic conditions in mineral medium using *C. crenatum* cells harvested from glutamic acid broth.

Conclusions

We investigated the feasibility of using cells harvested from glutamic acid fermentation broth for organic acid production under anaerobic conditions in mineral medium. *C. crenatum* could be used to produce succinic acid and lactic acid at high cell density with sustained organic acid production rate for at least 100 h. The process was more efficient than some of the conventional fermentations in terms of volumetric productivity and dispensability of complex nutrients. Wheat bran hydrolysates can thus be used for organic acid production by *C. crenatum*, which will be beneficial to the comprehensive utilization of wheat bran. Further studies on *C. crenatum* genetically modified to produce succinic acid are needed.

Acknowledgments Financial support from the National Natural Science Foundation of China (No. 31071636/31101352) and Program of the Key Laboratory for Agriculture Products Processing of Anhui Province (2009AKSY0102) are gratefully acknowledged.

References

Bormann E, Eikmanns B, Sahm H (1992) Molecular analysis of the *Corynebacterium glutamicum gdh* gene encoding glutamate dehydrogenase. Mol Microbiol 6:317–326

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Bunch PK, Mat-Jan F, Lee N, Clark DP (1997) The *IdhA* gene encoding the fermentative lactate dehydrogenase of *Escherichia coli*. Microbiology 143:187

Cao Y, Ding J, Duan ZY, Liu LM, Shi ZP (2009) On-line glucose concentration prediction and control for improving glutamate fermentation performance. Microbiology 36:1619–1624

Danshina P, Schmalhausen E, Avetisyan A, Muronetz V (2001) Mildly oxidized glyceraldehyde-3-phosphate dehydrogenase as a possible regulator of glycolysis. IUBMB Life 51:309–314

Dominguez H, Nezondet C, Lindley N, Cocaign M (1993) Modified carbon flux during oxygen limited growth of *Corynebacterium glutamicum* and the consequences for amino acid overproduction. Biotechnol Lett 15:449–454

Guettler MV, Jain MK, Rumler D (1996) US Patent Patent No. 5,573,931

John RP, Nampoothiri KM, Pandey A (2007) Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives. Appl Microbiol Biotechnol 74:524–534

Li Z, Han L, Ji Y, Wang X, Tan T (2010) Fermentative production of L-lactic acid from hydrolysate of wheat bran by *Lactobacillus rhamnosus*. Biochem Eng J 49:138–142

Litchfield JH (1996) Microbiological production of lactic acid. Adv Appl Microbiol 42:45–95

Lu S, Eiteman MA, Altman E (2009) Effect of CO_2 on succinate production in dual-phase *Escherichia coli* fermentations. J Biotechnol 143:213–223

McKinlay JB, Vieille C (2008) ^{13}C-metabolic flux analysis of *Actinobacillus succinogenes* fermentative metabolism at different $NaHCO_3$ and H_2 concentrations. Metab Eng 10:55–68

Meynial-Salles I, Dorotyn S, Soucaille P (2008) A new process for the continuous production of succinic acid from glucose at high yield, titer, and productivity. Biotechnol Bioeng 99:129–135

Nigam J (2001) Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*. J Biotechnol 87:17–27

Okino S, Inui M, Yukawa H (2005) Production of organic acids by *Corynebacterium glutamicum* under oxygen deprivation. Appl Microbiol Biotechnol 68:475–481

Okino S, Noburyu R, Suda M, Jojima T, Inui M, Yukawa H (2008) An efficient succinic acid production process in a metabolically engineered *Corynebacterium glutamicum* strain. Appl Microbiol Biotechnol 81:459–464

Sauer M, Porro D, Mattanovich D, Branduardi P (2008) Microbial production of organic acids: expanding the markets. Trends Biotechnol 26:100–108

Song H, Lee SY (2006) Production of succinic acid by bacterial fermentation. Enzym Microb Technol 39:352–361

Vemuri G, Eiteman M, Altman E (2002) Succinate production in dual-phase *Escherichia coli* fermentations depends on the time of transition from aerobic to anaerobic conditions. J Ind Microbiol Biotechnol 28:325–332

Wang JY, Zhu SG, Xu CF (2002) Biochemistry. Higher Education Press, Beijing, pp 100–102

Wu H, Li Z, Zhou L, Ye Q (2007) Improved succinic acid production in the anaerobic culture of an *Escherichia coli pflB ldhA* double mutant as a result of enhanced anaplerotic activities in the preceding aerobic culture. Appl Environ Microbiol 73:7837–7843

Xu H, Dou WF, Xu HY, Zhang XM, Rao ZM et al (2009) A two-stage oxygen supply strategy for enhanced L-arginine production by *Corynebacterium crenatum* based on metabolic fluxes analysis. Biochem Eng J 43:41–51

Zeikus J, Jain M, Elankovan P (1999) Biotechnology of succinic acid production and markets for derived industrial products. Appl Microbiol Biotechnol 51:545–552

Zhang ZY, Jin B, Kelly JM (2007) Production of lactic acid from renewable materials by *Rhizopus fungi*. Biochem Eng J 35:251–263

Zheng P, Dong JJ, Sun ZH, Ni Y, Fang L (2009) Fermentative production of succinic acid from straw hydrolysate by *Actinobacillus succinogenes*. Bioresour Technol 100:2425–2429

Zhou S, Causey T, Hasona A, Shanmugam K, Ingram L (2003) Production of optically pure D-lactic acid in mineral salts medium by metabolically engineered *Escherichia coli* W3110. Appl Environ Microbiol 69:399

Zwicker N, Theobald U, Zahner H, Fiedler H (1997) Optimization of fermentation conditions for the production of ethylene-diamine-disuccinic acid by *Amycolatopsis orientalis*. J Ind Microbiol Biotechnol 19:280–285