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Efficient production of succinic acid from macroalgae hydrolysate by metabolically engineered *Escherichia coli*

Bing Bai [a,b], Jie-min Zhou [a,b], Mao-hua Yang [a], Yi-lan Liu [a,b], Xiao-hui Xu [a,b], Jian-min Xing [a,*]

[a] National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China

HIGHLIGHTS

- *E. coli* BS002 was created to produce succinic acid from *L. japonica* hydrolysate.
- Glucose and mannitol were two major fermentable sugars in hydrolysate.
- Mannitol was superior to glucose for succinic acid production due to higher yield.
- High titer and yield of succinic acid from macroalgae biomass were reported firstly.

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ABSTRACT

In this study, microbial production of succinic acid from macroalgae (i.e., *Laminaria japonica*) was investigated for the first time. The engineered *Escherichia coli* BS002 exhibited higher molar yield of succinic acid on mannitol (1.39 ± 0.01 mol/mol) than glucose (1.01 ± 0.05 mol/mol). After pretreatment and enzymatic hydrolysis, *L. japonica* hydrolysate was mainly glucose (10.31 ± 0.32 g/L) and mannitol (10.12 ± 0.17 g/L), which was used as the substrate for succinic acid fermentation with the recombinant BS002. A final 17.44 ± 0.54 g/L succinic acid was obtained from the hydrolysate after 72 h dual-phase fermentation. The yield was as high as 1.24 ± 0.08 mol/mol total sugar, which reached 73% of the maximum theoretical yield. The results demonstrate that macroalgae biomass represents a novelty and economical alternative feedstock for biochemicals production.

1. Introduction

Succinic acid, a C4 dicarboxylic acid, has been widely used in many fields, such as food, agriculture, pharmaceutical and polymer industries (Jang et al., 2012). Furthermore, it is a very important platform compound for bulk chemicals production like 1,4-butanediol, tetrahydrofuran, adipic acid and γ-butyrolactone (Cheng et al., 2012). Currently, succinic acid is mainly produced by conventional chemical routes. However, declining reserves of petroleum resource and severe environmental impacts have turned biological production of succinic acid into a dominated trend (McKinlay et al., 2007).

Escherichia coli has become an excellent succinate-producer because of many advantages, such as clear genomic information, available genetic tools, low nutrient requirements and rapid growth rate (Cao et al., 2011; Chen et al., 2013). A variety of modified strategies have been employed to improve succinate synthesis. Byproduct pathways were knocked out (i.e., lactate dehydrogenase and pyruvate-formate lyase) (Bunch et al., 1997). Key limited-reactions were enhanced (i.e., phosphoenolpyruvate carboxylase and phosphoenolpyruvate carboxykinase) (Tan et al., 2013; Wang et al., 2011). Exogenous synthetic routes were introduced (pyruvate carboxylase) (Sanchez et al., 2005). Cofactors regulation (Liang et al., 2012) and evolution engineering (Zhang et al., 2009; Zhu et al., 2014) were also studied. Generally, glucose or xylose was used as the substrate for the engineered *E. coli*. Meanwhile, some terrestrial biomass, such as sugarcane (Chan et al., 2012), cassava (Chen et al., 2014) and corn stalk (Jiang et al., 2014), were explored as alternative feedstocks for succinic acid production.

Macroalgae, which are called "the 3rd generation biomass", have been considered to be a promising renewable feedstock for biofuels and biochemicals (John et al., 2011). They exhibit some attractive features superior to land-based crops and lignocellulosic

* Corresponding author. Tel.: +86 10 62550913, +86 10 62520135; fax: +86 10 62561822.
E-mail address: jmxing@home.ipe.ac.cn (J.-m. Xing).

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materials. Cultivation of macroalgae does not require arable land, freshwater or fertilizers, which is crucial to avoid competition with food-crops (van Hal et al., 2014). Furthermore, they have stronger photosynthetic efficiency (6–8%) and higher biomass density (565 ton/ha) than those of terrestrial crops (1.8–2.2% and 180 ton/ha) (Xu et al., 2014). Additionally, owing to lack of hemicellulose and lignin, it only needs relatively simple pretreatment to release fermentable sugars from macroalgae, which substantially lowers the process costs (van Hal et al., 2014; Wei et al., 2013). Recently, alginate metabolism has been successfully constructed in *E. coli* and *Saccharomyces cerevisiae* by Yoshikuni and his colleagues (Enquist-Newman et al., 2014; Wargacki et al., 2012), which is a key bottleneck for all sugars utilization in macroalgae, including alginate, mannitol and glucose. Therefore, macroalgae are regarded as a new appealing biomass for fuels or chemicals production. However, most of related studies mainly focus on the production of biofuels or biodiesel. There are only two reports about the production of biochemicals (2,3-butanediol and lactate) from macroalgae biomass with the engineered *E. coli* (Mazumdar et al., 2014, 2013), which deeply weakens full-potentials of "the 3rd-generation biomass".

In the present study, the engineered *E. coli* BS002 was constructed and the feasibility of succinic acid production from *Laminaria japonica* (a typical macroalgae) was investigated. Two major components of *L. japonica* hydrolysate (mannitol and glucose) were explored for co-conversion to succinic acid in the dual-phase fermentation. To our knowledge, this is the first report about the investigation of succinic acid fermentation from macroalgae biomass with the engineered *E. coli*.

2. Methods

2.1. Strains and plasmids

Strains, plasmids and primers used in this study are presented in Table 1. Wild type *E. coli* K-12 strain W3110 was kindly provided by professor Wang (Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences) and all genetic modifications were conducted in this strain or its derivatives.

Chromosomal genes knockout were made following the procedures of one-step inactivation method (Datsenko and Wanner, 2000). Sense and anti-sense primers contained 50 bp upstream or downstream homologous sequences of the targeted genes followed by 20 bp corresponding sequences of the kanamycin cassette. The DNA fragment was amplified from pKD4 and electroporated into *E. coli* W3110 or its derivatives harboring pKD46 for homologous recombination. The kanamycin-resistant strain with the targeted gene deleted was screened and verified by colony PCR and DNA sequencing. The kanamycin cassette was subsequently excised from *E. coli* genome by pFT-A carrying FLP-recombinase. The helper plasmids pKD46 and pFT-A were removed at 42 °C.

2.2. Preparation of L. japonica hydrolysate

Based on the reported optimized results about pretreatments and enzymatic methods of algal biomass (Kim et al., 2011), *L. japonica* (Dalian, Liaoning province, China) was washed 2–3 times with distilled water, dried overnight at 60 °C, ground with a blender and sieved through a 40 mesh screen. Finally, the dried powder of *L. japonica* was collected and stored at 4 °C. 100 g of the powder and 1000 mL 0.1 N HCl were mixed and heated at 121 °C for 15 min. Then pH of acid hydrolysate was adjusted to 5.0 with 10 N NaOH. The crude cellulase (Ningxia Xiasheng Industry Ltd., China) including endo/exo-glucanase and β-glucanase was added at the ratio of 0.1 g/g of dried algal biomass. Enzymatic process was carried out at 50 °C on an orbital shaker at 150 rpm for 24 h and then filtered to remove any residual solid materials. Finally, the *L. japonica* hydrolysate was obtained with about 10.31 ± 0.32 g/L glucose and 10.12 ± 0.17 g/L mannitol, the yield of total sugar reached 0.20 ± 0.01 g/g of dried algal biomass.

2.3. Dual-phase fermentation on pure glucose or mannitol in the shaking flask

Luria-Bertani (LB) medium was used for aerobic cultivation. For anaerobic fermentation, LB broth was supplemented with 15 g/L glucose or mannitol. When needed, 50 mg/L kanamycin was added to the medium.

1 mL of seed inoculum from an overnight 10 mL culture was added into 250 mL flask containing 50 mL LB medium for aerobic growth at 37 °C and 200 rpm. With preliminary optimization of transitional time (Supplementary Fig. S1), anaerobic fermentation was carried out after 6 h of aerobic incubation (OD_{600} = 4.0). Cells were harvested by centrifugation and resuspended in 30 mL of fresh anaerobic LB medium containing 15 g/L glucose or mannitol. Then these cultures were transferred to 100 mL shaking flasks with 0.45 g $MgCO_3$ and placed at an anaerobic incubator (CIMO Medical Instrument Manufacturing Ltd., Shanghai, China) without shaking at 37 °C for 48 h. Samples at 0 h and 48 h in anaerobic phase were collected and stored for analysis of biomass, glucose, mannitol and

Table 1
Strains, plasmids and primers used in this study.

	Relevant characteristics	Source
Strains		
W3110	Wild type *E. coli* K-12	CGSC 4474
BS001	W3110Δ*ldhA*	This study
BS002	W3110Δ*ldhA*Δ*pflB*::Kan	This study
Plasmids		
pKD46	*bla* red recombinase expression vector, temperature-sensitive *ori*	Our lab
pKD4	*bla* FRT-*kan*-FRT	Our lab
pFT-A	*bla* FLP recombinase expression vector, temperature-sensitive *ori*	Our lab
Primers		
ldhA-up	AAATATTTTTAGTAGCTTAAATGTGATTCAACATCACTGGAGAAAGTCTTGTGTAGGCTGGAGCTGCTTC	This study
ldhA-down	ATTGGGGATTATCTGAATCAGCTCCCCTGGGTTGCAGGGGAGCGGCAAGAATGGGAATTAGCCATGGTCC	This study
ldhA-jF	CGGCACAAGAATAATCAGTAATAACAGCG	This study
ldhA-jR	GCGGCTACTTTCTTCATTGTGGTTCTC	This study
pflB-up	TTACGGGCCTATAAGCCAGGCGAGATATGATCTATATCAATTTCTCATGTGTAGGCTGGAGCTGCTTC	This study
pflB-down	ATGCAGTCTGAACGTATTTATTTGGTATGGGCCCATCCTCGTCATGATATGGGAATTAGCCATGGTCC	This study
pflB-jF	GAGGATGCGGAAAAAATTCAACTCATT	This study
pflB-jR	GGATAAGCCTAACTCATCGCCCG	This study

metabolites. All shaking flask fermentation experiments were performed in triplicate.

2.4. Dual-phase fermentation on sugar mixture and L. japonica hydrolysate in 5-L bioreactor

Dual-phase fermentation of the sugar mixture of modeled *L. japonica* hydrolysate was carried out in 5-L bioreactor (New Brunswick Scientific Co., Inc. USA) containing 3 L LB medium. In aerobic phase, 5% seed cultures were inoculated. The flow rate of sterile air was controlled at 5 L/min with 500 rpm of agitation speed. The temperature was kept at 37 °C. The anaerobic phase was started when OD_{600} reached 4.0, CO_2 gas was sparged with a flow rate of 2 L/min and agitation speed decreased to 200 rpm. Simultaneously, the concentrated mixture was gradually fed into the medium until the concentrations of glucose and mannitol reached 10 g/L, respectively. The pH was kept at about 7.0 with 10 M NaOH and 10% H_2SO_4. The dual-phase fermentation of actual *L. japonica* hydrolysate was performed with the same method as described above, 20 g/L initial total sugars from *L. japonica* hydrolysate were supplemented at transition time from aerobic phase to anaerobic phase. All bioreactor fermentation experiments were performed in triplicate.

2.5. Analytical methods

The OD_{600} was determined to monitor cell growth and correlated to the dry cell weight (DCW): DCW (g/L) = 0.36 × OD_{600}. Glucose, mannitol and fermentation products (succinate, lactate, acetate, formate and pyruvate) were quantified by high-performance liquid chromatography (HPLC) equipped with UV absorbance detector and refractive index detector (Agilent Technologies, USA). The Aminex HPX-87H ion-exchange column was used for analysis (Bio-Rad, USA) and operated at 50 °C with a mobile phase of 5 mM H_2SO_4 solution at a flow rate of 0.6 mL/min.

3. Results and discussion

3.1. Glucose utilization for succinic acid production with different engineered E. coli strains during dual-phase fermentation

Under anaerobic condition, only 5% of carbon flux is transformed into succinic acid in wild type *E. coli* while most of residual flux is converted to lactate, formate and acetate (Zhu et al., 2013). In order to increase succinic acid biosynthesis and eliminate undesired byproducts, *E. coli* BS002 was constructed by knocking out lactate dehydrogenase (*ldhA*) and pyruvate-formate lyase (*pflB*) (Fig. 1). However, this mutant could not utilize glucose and exhibited poor cell growth under exclusively anaerobic condition (Supplementary Table S1), which was as similar as Vemuri et al. (2002a) reported previously. This growth deficiency is mainly due to the inability to synthesize acetyl-CoA and intracellular redox imbalance, which is known to accumulate high levels of pyruvate and NADH (Singh et al., 2011).

To solve this problem, the dual-phase fermentation strategy was chosen for restoring glucose consumption and cell growth of this mutant, in which aerobic biomass generation followed by anaerobic product accumulation. It has been demonstrated that such dual-phase fermentation is beneficial to strengthen the reductive TCA cycle and activate the glyoxylate pathway for succinate production in *E. coli* (Vemuri et al., 2002a,b). After 48 h, as shown in Table 2, the metabolites distribution of wild type *E. coli* W3110 or BS001, in which succinic acid was not the main product (0.27 ± 0.01 mol/mol and 0.35 ± 0.01 mol/mol, respectively), was nearly same as the results of its exclusively anaerobic

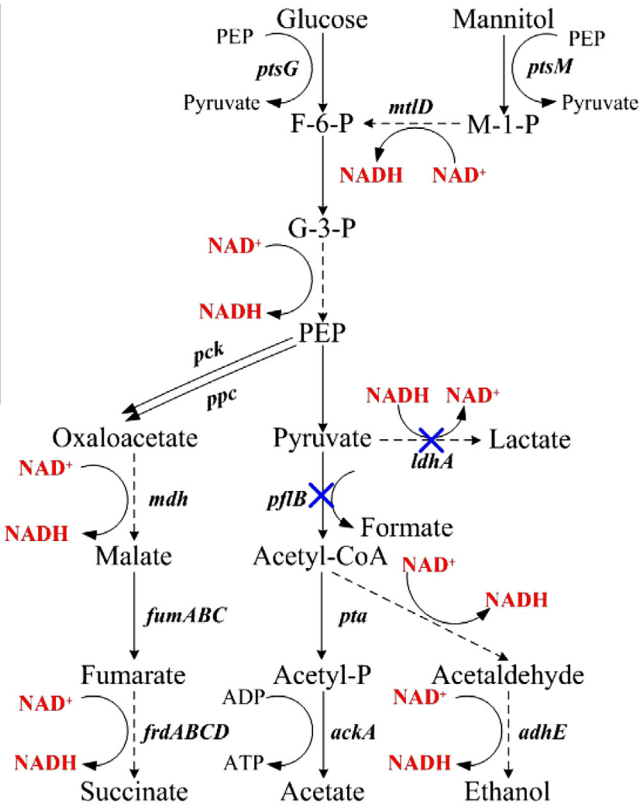

Fig. 1. Metabolic pathways of *Escherichia coli* in anaerobic fermentation on glucose and mannitol. Blue crosses represent gene deletions in present study. Dashed arrow represents the pathways involved generation of NADH/NAD$^+$. Genes and enzymes: *ptsG*, specific phosphoenolpyruvate phosphotransferase system for glucose; *ptsM*, specific phosphoenolpyruvate phosphotransferase system for mannitol; *mtlD*, mannitol-1-phosphate dehydrogenase; *pck*, phosphoenolpyruvate carboxykinase; *ppc*, phosphoenolpyruvate carboxylase; *mdh*, malate dehydrogenase; *fumABC*, fumarase isozymes; *frdABCD*, fumarate reductase; *ldhA*, lactate dehydrogenase; *pflB*, pyruvate-formate lyase; *pta*, phosphate acetyltransferase; *ackA*, acetate kinase; *adhE*, alcohol dehydrogenase. Abbreviations: F-6-P, fructose-6-phosphate; M-1-P, mannitol-1-phosphate; G-3-P, glyceraldehyde-3-phosphate; PEP, phosphoenolpyruvate; Acetyl-P, acetyl-phosphate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

fermentation. As expected, cell growth and glucose utilization of *E. coli* BS002 were regained through this two-stage approach. 14.58 ± 0.03 g/L glucose was consumed and 9.86 ± 0.48 g/L succinate was produced. The molar yield of succinic acid reached 1.01 ± 0.05 mol/mol, which was about 3.74-folds higher than other strains. Furthermore, only small amount of acetate (2.60 ± 0.14 g/L) was accumulated in fermentation broth. The results indicated that succinic acid could be efficiently produced from glucose with *E. coli* BS002 by the dual-phase fermentation.

3.2. Mannitol utilization for succinic acid production with different engineered E. coli strains during dual-phase fermentation

Mannitol, one of the major components of *L. japonica* hydrolysate, was used as a sole carbon source for succinic acid production. It is reported that more reducing powers drive more metabolic flux toward more reducing products (Liu et al., 2013a). Mannitol, with an oxidation state of −1, generates 1 mol NADH more than glucose per mole consumed (Fig. 1) and increases intracellular NADH availability on succinate synthesis.

This reducing sugar is delivered into the cytoplasm as mannitol-1-phosphate by a specific phosphoenolpyruvate phosphotransferase system (PTS) like glucose and then enters the glycolysis

Table 2
Dual-phase fermentation of different engineered strains in shaking flask fermentation with 15 g/L glucose.

Strains	DCW[a] (g/L)	Glucose consumed[a] (g/L)	Fermentation products [a] (g/L)				Yield[a] (mol/mol)
			Succinate	Lactate	Acetate	Formate	
W3110	3.44 ± 0.19	13.87 ± 0.10	2.47 ± 0.06	3.79 ± 0.42	3.76 ± 0.16	2.83 ± 0.28	0.27 ± 0.01
BS001	3.48 ± 0.07	14.12 ± 0.17	3.30 ± 0.10	ND[b]	5.17 ± 0.19	4.87 ± 0.24	0.35 ± 0.01
BS002	3.28 ± 0.04	14.58 ± 0.03	9.86 ± 0.48	ND	2.60 ± 0.14	ND	1.01 ± 0.05

[a] Each value is the mean of three parallel replicates ± standard deviation.
[b] ND not detected.

pathway as fructose-6-phosphate catalyzed by mannitol dehydrogenase (*mtlD*) (Solomon and Lin, 1972). After 48 h anaerobic phase on 15 g/L pure mannitol, as shown in Table 3, succinic acid in wild type *E. coli* W3110 and BS001 were only 2.83 ± 0.06 g/L and 3.13 ± 0.07 g/L, respectively. However, *E. coli* BS002 could convert 15.62 ± 0.01 g/L mannitol to 14.32 ± 0.09 g/L succinic acid, which was nearly 5-folds higher than other strains. The molar yield of succinic acid in this mutant reached up to 1.39 ± 0.01 mol/mol, which was 38% higher than that in glucose fermentation (1.01 ± 0.05 mol/mol). Furthermore, there was no accumulation of any by-products except 0.13 ± 0.02 g/L acetate. This phenomenon could be explained from two directions: (i) the deficiency of *ldhA* and *pflB* enforces succinate production become the only pathway to regenerate NAD^+; (ii) the metabolism of mannitol makes the intracellular redox more reducing than glucose. Similar results were also observed in *E. coli* CA102 and *Actinobacillus succinogenes* as these strains consumed sorbitol which was an isomeride of mannitol (Li et al., 2010; Liang et al., 2013).

Obviously, mannitol was a more preferable carbon source for succinate production when compared with glucose. These results also demonstrated that macroalgae biomass was superior to terrestrial biomass for succinic acid production as a result of high amounts of mannitol in these marine algae.

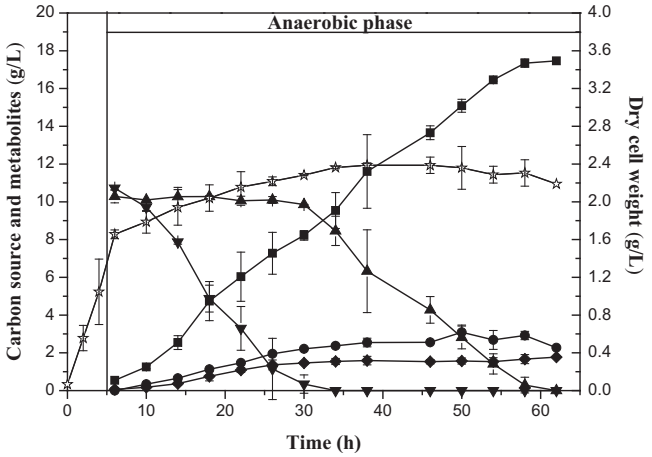

Fig. 2. Dual-phase fermentation of the modeled sugar mixture with *E. coli* BS002 in 5-L bioreactor. Symbols used in the figure: glucose (*filled triangle down*), mannitol (*filled triangle up*), succinic acid (*filled square*), pyruvate (*filled circle*), acetate (*filled diamond*), DCW (*empty star*). The error bars in the figure represent standard deviations from three parallel replicates.

3.3. Dual-phase fermentation of a sugar mixture of glucose and mannitol for succinic acid by the engineered E. coli BS002

Based on the actual ratio of *L. japonica* hydrolysate, a modeled mixture was constructed with 10 g/L glucose and 10 g/L mannitol. After 6 h aerobic growth, *E. coli* BS002 was transferred into exclusively anaerobic phase while the sugar mixture was also fed into the medium. As seen in Fig. 2, the engineered strain consumed glucose and mannitol completely in less than 56 h. However, a typical diauxic utilization pattern was exhibited between two sugars, which may be caused by catabolite repression of glucose on non-preferred carbon sources (Mazumdar et al., 2013).

During glucose-consumed stage, BS002 utilized 10.72 ± 0.09 g/L glucose entirely in the initial 24 h. The average sugar consumption rate was 0.45 ± 0.02 g/L/h, which was faster than that in mannitol-consumed stage (0.3 ± 0.01 g/L/h). 8.22 ± 0.26 g/L succinic acid was achieved, which comprised 46% of the total accumulated succinic acid. Additionally, a small amount of pyruvate (2.21 ± 0.02 g/L) and acetate (1.46 ± 0.08 g/L) was detected in the fermentation broth. Mannitol-consumed stage started after the depletion of glucose in the medium, 10.28 ± 0.33 g/L mannitol was efficiently metabolized within nearly 32 h. Succinic acid became the main metabolite accumulated in BS002 and increased by 9.24 ± 0.25 g/L during this stage. The molar yield was 1.36 ± 0.04 mol/mol, which was 21% higher than that in glucose-consumed phase. Meanwhile, the concentrations of pyruvate (2.91 ± 0.22 g/L) and acetate (1.67 ± 0.24 g/L) did not practically increase with the consumption of mannitol.

Finally, the overall succinic acid concentration reached up to 17.32 ± 0.22 g/L with the molar yield of 1.26 ± 0.03 mol/mol total sugar. The successful utilization of the sugar mixture for succinic acid production with the recombinant BS002 laid the foundation for *L. japonica* hydrolysate fermentation.

3.4. Dual-phase fermentation of the L. japonica hydrolysate for succinic acid by the engineered E. coli BS002

Unlike terrestrial biomass, *L. japonica* hydrolysate does not contain fermentation inhibitors such as 5-hydroxymethyl furfural

Table 3
Dual-phase fermentation of different engineered strains in shaking flask fermentation with 15 g/L mannitol.

Strains	DCW[a] (g/L)	Mannitol consumed[a] (g/L)	Fermentation products[a] (g/L)				Yield[a] (mol/mol)
			Succinate	Lactate	Acetate	Formate	
W3110	3.36 ± 0.03	16.21 ± 0.28	2.83 ± 0.06	3.60 ± 0.07	3.31 ± 0.08	4.89 ± 0.15	0.26 ± 0.01
BS001	3.34 ± 0.02	15.22 ± 0.61	3.13 ± 0.07	ND[b]	3.36 ± 0.09	6.20 ± 0.05	0.31 ± 0.01
BS002	2.96 ± 0.01	15.62 ± 0.01	14.32 ± 0.09	ND	0.13 ± 0.02	ND	1.39 ± 0.01

[a] Each value is the mean of three parallel replicates ± standard deviation.
[b] ND not detected.

Table 4
Dual-phase fermentation of the engineered *E. coli* BS002 in shaking flask fermentation with different carbon sources.[c]

Carbon source	DCW[c] (g/L)	Glucose consumed[c] (g/L)	Mannitol consumed[c] (g/L)	Fermentation products[c] (g/L)			Yield[c](mol/mol)
				Succinate	Pyruvate	Acetate	
Medium A[a]	3.22 ± 0.04	4.90 ± 0.04	5.27 ± 0.04	8.32 ± 0.06	0.43 ± 0.05	1.81 ± 0.17	1.23 ± 0.01
Medium B[b]	3.16 ± 0.09	4.14 ± 0.10	5.20 ± 0.20	8.11 ± 0.21	0.36 ± 0.04	1.94 ± 0.06	1.31 ± 0.01

[a] Medium A represents the sugar mixture was added into LB medium.
[b] Medium B represents *L. japonica* hydrolysate was added into LB medium.
[c] Each value is the mean of three parallel replicates ± standard deviation.

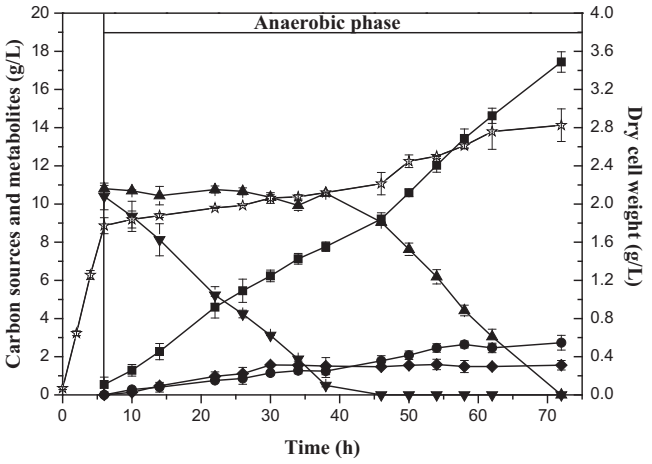

Fig. 3. Dual-phase fermentation of *L. japonica* hydrolysate with *E. coli* BS002 in 5-L bioreactor. Symbols used in the figure: glucose (*filled triangle down*), mannitol (*filled triangle up*), succinic acid (*filled square*), pyruvate (*filled circle*), acetate (*filled diamond*), DCW (*empty star*). The error bars in the figure represent standard deviations from three parallel replicates.

(HMF), soluble phenolic compounds due to the absence of lignin and mild pretreatment conditions (Ge et al., 2011). However, it was not clear whether *L. japonica* might generate some other unknown by-products during pretreatment and hydrolysis, which played an inhibitory effect on subsequent microbial fermentation.

For preliminary evaluation of the hydrolysate as a carbon source, dual-phase fermentation was carried out in the shaking flasks firstly. The concentrated sugar mixture and hydrolysate were added to fermentation medium with about 10 g/L initial total sugars. As shown in Table 4, BS002 could consume glucose (4.14 ± 0.10 g/L) and mannitol (5.20 ± 0.20 g/L) in *L. japonica* hydrolysate completely. The cell density was nearly 3.16 ± 0.09 g/L. Succinate concentration finally reached 8.11 ± 0.21 g/L with a molar yield of 1.31 ± 0.01 mol/mol, which was even slightly higher than that (1.23 ± 0.01 mol/mol) in sugar mixture fermentation. These results indicated that *L. japonica* hydrolysate could be utilized effectively for succinic acid production without obvious inhibition.

Furthermore, in order to confirm fermentation properties of *L. japonica* hydrolysate as the substrate, dual-phase fermentation was also conducted in 5-L bioreactor. The initial total sugar concentration was maintained at about 20 g/L with 10.40 ± 0.70 g/L glucose and 10.81 ± 0.17 g/L mannitol. As seen in Fig. 3, the hydrolysate fermentation exhibited the same sugar utilization pattern as the modeled sugar mixture. However, the consumption rate of glucose in hydrolysate was 30% lower than that in pure mixture. It seemed that small amounts of formic acid or acetic acid formed in hydrolysis process decreased sugar consumption in subsequent fermentation (Wang et al., 2011). In the meantime, the ability of uptaking mannitol remained nearly same. This was likely due to higher cell density (2.83 ± 0.17 g/L DCW) in hydrolysate than that (2.30 ± 0.14 g/L DCW) in the modeled mixture during the later period of fermentation process. The improved cell growth could be explained by the fact that actual hydrolysate contained abundant nutrients, such as nitrogen or minerals. After 72 h, glucose and mannitol got completely utilized and 17.44 ± 0.54 g/L succinate were produced. Similarly, the detectable by-products were pyruvate (2.73 ± 0.38 g/L) and acetate (1.55 ± 0.25 g/L), respectively. The final molar yield of *L. japonica* hydrolysate fermentation was 1.24 ± 0.08 mol/mol total sugar, which was comparable with some results of succinate production on terrestrial biomass by the engineered *E. coli* (Chen et al., 2014; Jiang et al., 2014; Liu et al., 2013b; Wang et al., 2011).

Concerned genetic characteristics of *E. coli* BS002 (Δ*ldhA*Δ*pflB*), higher molar yield and stronger productivity of succinic acid on macroalgae biomass could be expected by further design and modification on this strain.

4. Conclusions

The engineered *E. coli* BS002 was constructed for succinic acid production from *L. japonica* hydrolysate. As a result, after 72 h batch dual-phase fermentation, the concentration and molar yield of succinic acid in BS002 were 17.44 ± 0.54 g/L and 1.24 ± 0.08 mol/mol total sugar, respectively. It was found that the molar yield of succinic acid on mannitol was significantly higher than that on glucose due to more NADH supply. With abundant mannitol in hydrolysate, macroalgae show great potentials on biochemicals production, especially for reductive chemicals, such as succinate, malate and fumarate.

Acknowledgements

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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.2015.02.081.

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