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Production of xylitol by expressing xylitol dehydrogenase and alcohol dehydrogenase from *Gluconobacter thailandicus* and co-biotransformation of whole cells

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ABSTRACT

In the present study, recombinant strains were constructed for xylitol production by cloning and expressing the novel xylitol dehydrogenase (*xdh*) and alcohol dehydrogenase (*adh*) genes in *E. coli* BL21 (DE3) from *Gluconobacter thailandicus* CGMCC1.3748. The optimum pH, temperature, specific activity and kinetic parameters were further investigated for purified XDH. The co-culture of *G. thailandicus* (30 g/L), BL21-*xdh* (20 g/L) and BL21-*adh* (20 g/L) produced 34.34 g/L of xylitol after 48 h in the presence of 40 g/L D-arabitol and 2% ethanol. The concentration of xylitol produced in this co-biotransformation was found to be 2.7-folds higher than the xylitol yield of *G. thailandicus* alone, while the yield was increased by 4.8% when compared to that of *G. thailandicus* mixed with BL21-*xdh* under the similar experimental conditions.

1. Introduction

Xylitol is a naturally occurring pentahydroxy sugar alcohol, which has a wide variety of applications in food and pharmaceuticals industries (Dasgupta et al., 2017; de Albuquerque et al., 2015). Since xylitol provides rapid sweetness, flavor, quick cooling effect and as a low-energy sweetener, it has been popularly used in the formulation of various food and confectionery products, such as sugar-free chewing gum and bakery products. Additionally, xylitol can be used as a sweetener in many pharmaceutical preparations as a substitute of sucrose (Jia et al., 2016). Xylitol-sweetened medications are used to prevent dental caries as it causes very little increase in glucose level and insulin in blood (Cardoso et al., 2016). Currently lignocellulosic biomass based biorefineries produced a variety of value added products, where xylitol has been placed as one of the 12 important value-added products by the US Department of Energy (Ghaffar et al., 2017; Venkateswar Rao et al., 2016). Due to the growing demand of xylitol in food, pharmaceuticals and odontological industries, consumption of xylitol has been increased dramatically in recent years, which was roughly 160,000 metric tons in 2013 and is expected to be 242,000 metric tons by 2020 (Rao et al., 2016). Likewise, the market value of xylitol is also rising significantly day by day as estimated for US$ 670 million in 2013 to US$ 1 billion by 2020 with the selling price of US $ 4.5–5.5/kg for bulk in industries and US$ 20/kg as the final product in the supermarkets (Rao et al., 2016; Ravella et al., 2012).

Although there is a growing interest in the biological production of xylitol from renewable materials (Albuquerque et al., 2014; Dhar et al., 2016), at present, most of the xylitol is produced on commercial scale by traditional chemical approach that include hydrogenation of pure D-xylose derived from the biomass (Ur-Rehman et al., 2015). Even though D-xylose can be obtained from cheap lignocellulosic (de Albuquerque et al., 2015; Zabed et al., 2016), the traditional chemical route of xylitol production is very costly due to some drawbacks and economic issues. Firstly, it requires high cost Raney-nickel catalyst together with high pressure (10–15 atm) and elevated temperature of up to 130 °C (Cheng et al., 2014). Moreover, chemical synthesis of xylitol employs not only the expensiveness process, but also requires high costs and high amounts of water for purification of xylose from hemicellulose hydrolysates, in addition to the requirement of costly refining process for downstream xylitol recovery (Albuquerque et al., 2014; Pal et al., 2016). What is more, chemical synthesis of xylitol is not environmentally friendly and is energy consuming (Dasgupta et al., 2017; Li et al., 2015).

Alternatively, microbial biotransformation of xylose into xylitol has

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been investigated using several yeast strains such as *Debaryomyces hansenii*, *Candida tropicalis* and *Candida parapsilosis* (Li et al., 2016). This bioconversion route is further found to be challenging because of the requirement of hydrolysis of hemicellulose and purification of xylose from the hydrolysate as it contains microbial inhibitors (Li et al., 2016). Compared to xylose, glucose could be a good candidate for xylitol production in the perspectives of the technology and economy (Mayer et al., 2002). However, natural microorganisms cannot convert glucose to xylitol directly due to the lack of comprehensive metabolic pathway and hence, it is necessary to employ a two-step biotransformation process, in which glucose is first converted into D-arabitol by yeasts that undergoes subsequent biotransformation into xylitol by microorganisms (Li et al., 2016; Qi et al., 2014). This two-step biotransformation system is therefore technologically complex and time consuming. As a result, although D-arabitol is a relatively costly substrate compared to glucose, it has some promising advantages and can be used for xylitol production by the most efficient xylitol producing strains, such as *Gluconobacter* sp., which do not have the metabolic system to convert glucose into D-arabitol, in addition to the fact that utilization of D-arabitol as substrate reduces process step and risk of contamination (Zhang et al., 2013; Qi et al., 2015). Additionally, D-arabitol can be produced from various renewable sources such as glucose, glycerol and xylose by simple microbial biotransformation (Kordowska-Wiater, 2015; Yoshikawa et al., 2014; Zhu et al., 2010).

Two key enzymes play important roles in the bioconversion of xylitol from D-arabitol, namely D-arabitol dehydrogenase (ArDH) and NADH-dependent xylitol dehydrogenase (XDH), respectively (Qi et al., 2014). In an earlier study, we developed a high xylitol yield strain by expressing *xdh* in *E. coli* BL21 from *Gluconobacter oxydans* CGMCC1.49, where the mixed culture of recombinant and *G. oxydans* strains produced 0.83 g/g of xylitol from D-arabitol in the presence of exogenous NADH (Qi et al., 2016). In another study, we have constructed two recombinant strains, namely BL21-*xdh* and BL21-*ardh* that contained novel *xdh* and *ardh*, respectively, from *Gluconobacter* sp. JX-05 and produced 26.1 g/L of xylitol with a yield of 0.87 g/g in a mixed culture of both strains using exogenous NADH (Qi et al., 2017).

However, the recombinant strains developed in the above studies were devoid of system of regenerating NADH by the cells themselves even though sufficient supply of NADH is required for the activity of XDH during bioconversion of xylulose to xylitol. It has resulted the requirement of adding a good quantity of exogenous NADH, which does not offer a cost-effective bioconversion system. Therefore, a cofactor regeneration system in the recombinant cell factory is very important for enhanced xylitol yield from D-arabitol and co-factor self-sufficiency of whole cell biocatalysis (Jo et al., 2015; Wang et al., 2017b). One of the most convenient ways to produce indigenous NADH by the cells is the introduction of *adh* in the cell factory along with the addition of a co-substrate, such as ethanol (Weckbecker et al., 2010; Zhou et al., 2012). Therefore, a recombinant *E. coli* BL21 (DE3) was developed in this study by cloning and expressing a novel *adh* from *G. thailandicus* CGMCC1.3748 that generated the recombinant BL21-*adh* strain. Likewise, a novel *xdh* was also cloned from *G. thailandicus* to construct the recombinant BL21-*xdh*. Both *adh* and *xdh* strains were studied for whole cell co-biotransformation of D-arabitol along with the original *G. thailandicus* strain in single and mixed cultures for xylitol production.

2. Materials and methods

2.1. Strain, plasmid, culture medium and conditions

E. coli BL21 (DE3) (Stratagene, USA) was used as the host strain for heterologous gene expression. The expression plasmid used in this study was pET-30a (+) (Invitrogen, USA). *G. thailandicus* CGMCC1.3748, a xylitol producing strain, was obtained from the China General Microbiological Culture Collection Center (CGMCC, China). *E. coli* strains were grown aerobically in LB broth supplemented with 100 μg/mL of Kanamycin (LB-Kan) at 160 rpm for 12 h. *G. thailandicus* CGMCC1.3748 was grown in the medium composed of Glucose (100 g/L), Yeast extract (10 g/L), and $CaCO_3$ (20 g/L). Cultivation was carried out aerobically in a shaking incubator at 30 °C and 150 rpm for 48 h.

2.2. Cloning of xdh and adh

The primers for *xdh* and *adh* genes used in this study were selected based on sequence of *G. thailandicus* from NCBI and characteristics of restriction sites of expression vector pET30a (+). The primes were as follows: *xdh*-1: 5-GTACCGACGACGACGACAAGATGGCATACGTGGTAAGTTC-3 (the *Nco*I site is underlined); *xdh*-2: 5-CTCGAGTGCGGCCGCAAGCTTCAGCCTCCGGAGATTTCCA-3 (the *Hind*III site is underlined); *adh*-1: 5-GTACCGACGACGACGACAAGATGTTTCGTCGTATCGTGCCCG-3 (the *NcoI* site is underlined); *adh*-1: 5-CTCGAGTGCGGCCGCAAGCTTCAGAGAAGCGGTTGAGCGG (the *Hind*III site is underlined). The cloning of *xdh* and *adh* genes were done by PCR with a denaturation step at 95 °C for 5 min, followed at 94 °C for 30 s, 55 °C for 30 s, and extension at 72 °C for 1 min, with final extension for 10 min at 72 °C with a total of 35 cycles, respectively. Subsequently, the plasmids of pET-30a (+) were digested with restricted enzyme of *Nco*I and *Hind*III , and the PCR fragments of *xdh* and *adh* were linked to pET30a (+) separately using One Step Cloning Kit. The recombinant plasmid named as pET-*xdh* and pET-*adh* were confirmed by PCR test and sequencing by Genscript Biotech (Nanjing, China).

2.3. Expression of xdh and adh

The recombinant plasmids, pET-*xdh* and pET-*adh*, were transferred into the competent cells of *E. coli* BL21 (DE3) separately by the One Step Cloning Kit, and the recombinant strains thus obtained were named as BL21-*xdh and* BL21-*adh*, respectively. Thereafter, BL21-*xdh* and BL21-*adh* recombinant strains were cultivated separately in Erlenmeyer flasks containing LB-Kan medium at 37 °C for 8–12 h. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was supplemented with the final concentration of 1.0 mM when the optical density of the culture broth at 600 nm was about 0.4–0.6. After supplementation, cells were allowed to grow under low temperature (16 °C) for 20 h. Then the culture broth was centrifuged for 15 min at 8000 rpm and washed twice with 0.1 M potassium phosphate buffer (100 mM potassium phosphate, 300 mM potassium chloride, pH 7.4). The re-suspended cells were disrupted by ultrasonication (250 W, working 2 s and intervening 2 s), followed by centrifugation for 15 min at 8000 rpm. Supernatant was collected as the crude enzyme.

2.4. Purification of XDH

The crude XDH mentioned above was purified by nickel affinity chromatography for further characterization using the modified method described earlier (Qi et al., 2017). Briefly, the crude enzyme was passed through a 0.22 μm filter and then loaded onto Ni-NTA column equilibrated with binding buffer for 5 mM imidazole (100 mM potassium phosphate and 300 mM KCl buffer and 5 mM imidazole). The column was eluted with 45 mM imidazole to remove non-target proteins and further eluted the column with 200 mM imidazole to collect purified XDH. The purified enzyme was dialyzed with Tris-HCl buffer (50 mM, pH 7.4) and identified by 12% SDS-PAGE. The protein concentration in purified enzyme was determined by the Bradford method. All purification steps were carried out at 4 °C.

2.5. Enzyme assay

The oxidation and reduction activity of XDH was monitored spectrophotometrically at 340 nm by the initial rate of substrate-dependent NAD (H) consumption and production, respectively. The effects of pH on enzyme activity was determined under different pH levels using

0.1 M potassium acetate buffer (pH 4–6), 0.1 M potassium phosphate buffer (pH 7–8), and 0.1 M Glycine-NaOH buffer (pH 9–12). The optimum reaction temperature was determined by investigating the enzyme activity at different temperatures ranging from 25 to 55 °C. The enzyme kinetics was analyzed by the software of Origin 8.0. Nonlinear regression equation of Michaelis-Menten was used for curve-fitting. One unit of enzyme activity was defined as the amount enzymes required to produce 1 μmol xylitol per min under the specified conditions.

2.6. Xylitol production by co-biotransformation

G. thailandicus CGMCC1.3748, BL21-*xdh*, and BL21-*adh* were used together for whole cells co-biotransformation. The method for preparing whole cells was carried out according to our previously method (Yun et al., 2018). Optimization of substrate concentration of D-arabitol for bioconversion was determined between 20 and 50 g/L. The mass ratio of *G. thailandicus* CGMCC1.3748, BL21-*xdh*, and BL21-*adh* between 20:20:20, 30:20:20, 20:30:20, and 20:20:30 was used to optimize for bioconversion. The whole process of co-biotransformation was carried out aerobically in 1 L shake flask with 400 mL D-arabitol (40 g/L D-arabitol) at 37 °C and 150 rpm.

2.7. Analytical methods

D-Arabitol, D-xylulose and xylitol in samples were detected by HPLC analysis (Agilent 1260) system equipped with refractive index detector and an Ultimate XB-NH2 HPLC Column (Welch, China), with 80:20 (v/v) mixture of deionized water and acetonitrile, as mobile phase. The flow rate was 0.8 mL/min. The supernatant was filtered through a 0.22 um membrane filter and 20 μL of a sample was then injected directly on to the HPLC column, and the column temperature 50 °C (Cortez et al., 2016).

3. Results and discussion

3.1. Cloning, expression and purification

The *xdh* and *adh* genes of *G. thailandicus* were amplified by PCR, which produced a 0.8 kb fragment and a 0.4 kb fragment, respectively. The two genes were subsequently recombined with separate plasmids and confirmed through restriction digestion and PCR analysis. The sequencing of both genes reveled that *xdh* and *adh* encoded 266 and 134 amino acids, respectively.

The recombinant strain of BL-*xdh* and BL-*adh* were induced by IPTG at a concentration of 1 mM at a low temperature (16 °C) to avoid the formation of inclusion body. The expressed XDH and ADH were analyzed by SDS–PAGE. Two separate protein brands were observed with molecular masses of about 33 kDa for XDH (Fig. 1) and 19 kDa for ADH

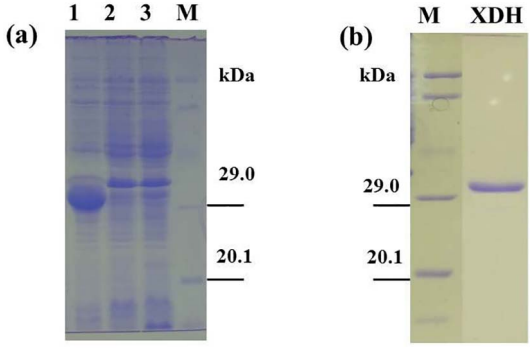

Fig. 1. SDS-PAGE analysis of XDH. (a) The crude extraction containing XDH, Lane M: protein marker, Lane 1: XDH from BL21-*xdh* with Isopropyl β-D-1-thiogalactopyranoside (IPTG) as inducer, Lane 2 and 3: XDH from BL21-*xdh* without IPTG. (b) The purified XDH, Lane M: protein marker, laneXDH: purified protein by Ni-NTA.

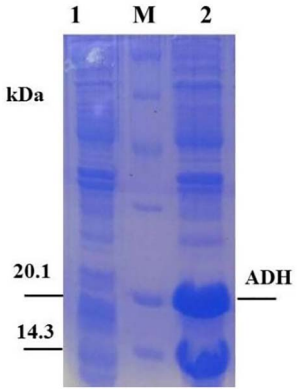

Fig. 2. SDS-PAGE analysis of ADH. The crude extraction containing ADH, Lane M: protein marker, Lane 1: BL21-*adh* without IPTG, Lane 2: ADH from BL21-*adh* with IPTG.

(Fig. 2), which revealed that both genes were expressed successfully in the recombinant strains. As the theoretical weight of XDH and ADH are 29 kDa and 15 kDa, respectively, the higher masses obtained for these two proteins after SDS-PAGE were due to the apparent size of fusion protein as migrated on the SDS-PAGE that included His-tag, S-Tag and enterokinase.

3.2. Effects of pH and temperature on XDH activity

The effect of pH on XDH activity was investigated by conducting reaction with enzymes, substrate and co-factor (NADH and NAD^+). The optimal pH for oxidation of D-xylulose in the presence of NADH was found to be around 9.0, while the best result of reduction of D-xylitol was observed at pH 6.0 in the presence of NAD^+ (Fig. 3a). However, the relative activity of XDH, particularly during oxidation, decreased significantly with the increase or decrease of the pH from the optimum, which were more apparent and sharp at the pH greater than 11. On the other hand, the relative activity of reduction was above 80% when pH ranged between 4.0 and 7.0.

The optimum pH for the oxidation activity of XDH found in this study is, however, slightly lower than the optimum pH found in the earlier studies for the same reaction of XDH isolated from two different strains of *G. oxydans*, where optimum pH for the oxidation activity of XDH were reported for 11.0 (Sugiyama et al., 2003) and 10 (Lin et al., 2012). On the other hand, a higher optimum pH was recorded for the reduction activity of XDH in this study when compared with that of reported values in the earlier studies (Lin et al., 2012). The reason of the variation in the optimum pH of XDH activity could be due to the isolation of enzyme from different source strains. Despite the variation in the optimum pH, the XDH of this study efficiently converted D-xylulose into xylitol.

The effect of temperature on the activity of XDH was also investigated in the range of 25–55 °C (Fig. 3b). The optimum temperature for the oxidation of D-xylulose was found to be 35 °C, while the optimum reduction of xylitol was observed at 30 °C. The relative activity of oxidation was above 80% at the temperature ranged from 25 to 35 °C. However, the activity dropped sharply as the temperature increased to 45 °C and even reached to only 23.6% at 55 °C. Likewise, the relative activity of reduction was decreased from 98.7% at 30 °C to only 14.4% at 55 °C.

3.3. Specific activities and kinetic analysis

The substrate specificity and kinetic properties of XDH were investigated using different substrates under the optimal pH and temperature, and the results are presented in Table 1. The results of the reduction reactions indicated that the specific activity of XDH for xylitol was the highest among those of D-mannitol, D-sorbitol and ethanol.

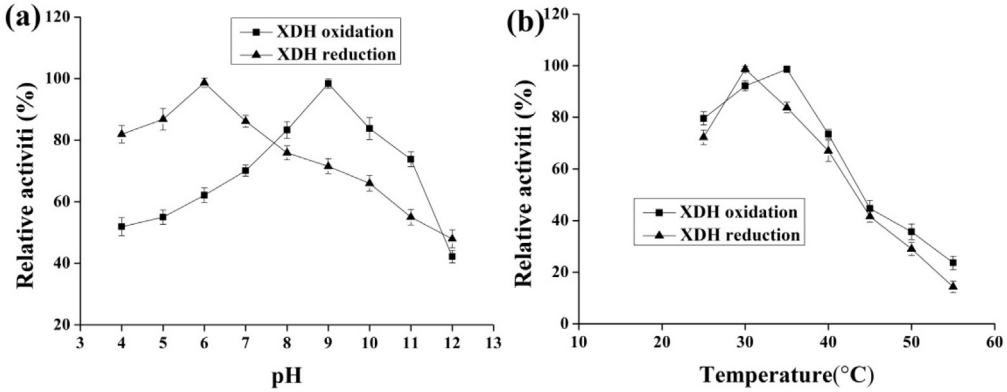

Fig. 3. The effects of pH and temperature on XDH activities. (a) pH and (b) temperature. The relative activity of XDH under the optimum pH and temperature was 100%.

Table 1
Specific activities of xylitol dehydrogenase (XDH) for various substrates.

Substrate	Specific activity (U/mg)	Relative activity (%)
Ehanol	4.2	25.6
Xylitol	16.6	100
D-Sorbitol	10.7	64.5
D-Mannitol	14.4	86.4
D-Xylulose	50.2	100
D-Fructose	30.1	60.0

Furthermore, the specific activity of XDH for D-xylulose was found to be about 1.6 folds higher than that of D-fructose during oxidative reactions. Therefore, D-xylulose was the preferred substrate to D-fructose for oxidative reactions of XDH, while xylitol was the preferred substrate for reduction reaction. On the other hand, the specificity activity of D-xylulose was about 3-folds higher than that of xylitol. As a result, it is supposed to be the best to generate xylitol by the oxidation than the reduction of D-xylose in this biotransformation. The substrate specificity was found to be consistent with the corresponding enzyme of *G. oxydans* NH-10 (Zhang et al., 2013).

During the enzyme assays, the kinetic parameters of XDH for xylitol was determined in the presence of NAD^+ under the optimum pH (6.0) at 30 °C, while the kinetic parameters for D-xylulose was determined with NADH at pH 9.0 and temperature 35 °C. Here, the XDH showed the classical Michaelis Menten kinetics with the K_m values 20.7 mM for D-xylulose, and 63.3 mM for xylitol, and with the V_{max} values 98.6 U/mg for D-xylulose and 39.0 U/mg for xylitol (Table 2). The results revealed that XDH has a higher affinity for D-xylulose than xylitol. The K_m value for NADH was found to be 4.2 mM with D-xylulose, and for NAD^+ was 14.1 mM with xylitol. Therefore, XDH has a higher affinity to NADH than NAD^+. The results of the kinetic parameters of XDH observed in this study were found to be similar to those reported in earlier studies with *G. oxydans* NH-10 (Zhang et al., 2013) and *G. oxydans* CGMCC 1.49 (Qi et al., 2016).

3.4. D-Xylitol production

Several key factors affected the production of xylitol during biotransformation. Firstly, as discussed above (Section 3.3), XDH is highly specific for NADH during oxidation of xylitol from D-xylulose, but it needed a sufficient supply of the NADH. Therefore, 2% ethanol was added to provide abundant NADH through catalysis activity of ADH as also described by Zhou et al. (2012). On the other hand, the concentration of D-arabitol also played an important role in xylitol yield. Therefore, the optimization of D-arabitol concentration was investigated using different concentrations of D-arabitol ranged between 20 and 50 g/L. After 12 h of biotransformation, the concentration of xylitol was found to be increased with the increase in the concentration of D-arabitol with obtaining the highest concentration of xylitol with 40 g/L of D-arabitol (Table 3). Hence, 40 g/L of D-arabitol was used in the subsequent experiments. The effect of *G. thailandicus* to BL21-*xdh* to BL21-*adh* mass ratio was also investigated. The results demonstrated that using 30 g/L of *G. thailandicus* CGMCC1.3748 mixed with 20 g/L BL21-*xdh* and 20 g/L BL21-*adh* was the optimum mass ratio for xylitol production during 12 h biotransformation (Table 3).

The natural strain of *G. thailandicus* produced 12.55 g/L of xylitol with a yield of 0.31 g/g after 30 h using 40 g/L of D-arabitol as the substrate and 2 g/L of ethanol as the co-substrate (Fig. 4a). On the other hand, the mixed culture consisting of 30 g/L of *G. thailandicus* CGMCC1.3748, 20 g/L of BL21-*xdh* and 20 g/L of BL21-*adh* produced 34.34 g/L of xylitol after 48 h with the maximum yield of 0.86 g/g at 36 h, which was almost 2.7-folds greater than that of *G thailandicus* CGMCC1.3748 alone (Fig. 4b). The mixed culture of *G. thailandicus* CGMCC1.3748 and 20 g/L BL21-*xdh* produced 32.80 g/L xylitol after 48 h of biotransformation with the yield of 0.82 g/g at 36 h, resulting in an increase in xylitol yield by 2.6 folds compared with the yield of *G. thailandicus* CGMCC1.3748 alone, even though the productivity was slightly lower than that of mixed culture of three strains (Fig. 4c).

In this study, xylitol yield (0.86 g/g) by the co-culture of three strains was found to be almost similar to the yield reported in our previous study (0.87 g/g) using the same substrate but different strains, even though no exogenous NADH was used in the present work unlike the previous work (Qi et al., 2017). On the other hand, xylitol yield of

Table 2
Characteristics of the recombinant xylitol dehydrogenase (XDH) based on different kinetic parameters determined for different substrates.

Substrate	K_m (mM)	V_{max} (U/mg)
D-Xylulose	20.7	132.8
Xylitol	63.3	98.6
NADH	4.2	55.6
NAD^+	14.1	39.0

Table 3
Effects of D-arabitol concentrations and mass ratios of strains on xylitol production during whole cell biotransformation in mixed culture (Results are shown as average ± SD).

Name of the factors	Level	Xylitol concentration (g/L)
D-Arabitol (g/L)	20	7.9 ± 0.13
	30	8.6 ± 0.07
	40	9.1 ± 0.28
	50	8.9 ± 0.35
Mass ratio (*G. thailandicus*: BL21-xdh: BL21-adh)	1:1:1	10.7 ± 0.20
	1.5:1:1	12.6 ± 0.11
	1:1.5:1	11.3 ± 0.32
	1:1:1.5	10.2 ± 0.24

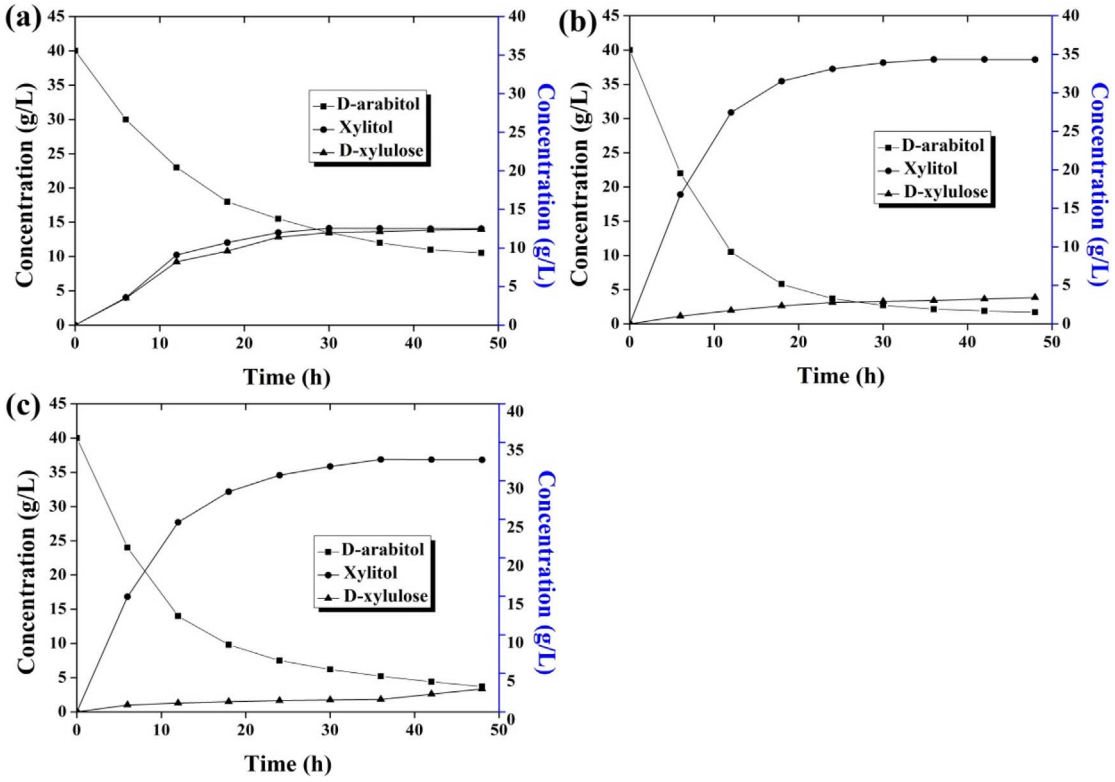

Fig. 4. Production of xylitol from D-arabitol by the original and recombinant strains. (a) *G. thailandicus* alone, (b) *G. thailandicus* mixed with BL21-*xdh* and BL21-*adh* and (c) *G. thailandicus* mixed with BL21-*xdh*.

the present work was higher than the yield reported in another study as 0.84 g/g of xylitol from the same substrate (Qi et al., 2016). The yield and productivity of xylitol in this study were also found to be higher than those reported in earlier using mixed cultures *E. coli* Rosetta/Duet-*xdh-adh* system (xylitol concentration 24.9 g/L, and yield 85.2%) (Zhou et al., 2012), and by using mixed cultures of *G. oxydans-xdh-gdh* system (xylitol concentration 12.23 g/L and yield 40.8%) (Zhang et al., 2013). In another study, a recombinant strain of *G. oxydans* PZ was developed by overexpressing glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH), which produced around 29.3 g/L of xylitol from D-arabitol, with 73.2% yield (Li et al., 2016).

Since the efficiency of biocatalysts will be limited by the insufficient cofactor supply, abundant cofactor is thus required for the effective biotransformation (Wang et al., 2015, 2017a,b). To overcome the shortage of co-factors, a self-sufficient system was constructed in the present study, where alcohol dehydrogenase was found to be played a crucial role in the cofactor regeneration. The regenerated NADH resulted enhanced metabolic shift toward reduction reaction, which in turn improved the production of xylitol. So, no external cofactor or redox source was used in this co-biotransformation. However, under this condition some byproducts (*e.g.*, acetaldehyde) were also produced, which require a further effort to remove or reduce formation of by-product.

4. Conclusion

In this study, the novel *xdh* and *adh* genes were successfully cloned and expressed to develop recombinant strains. The whole cell co-biotransformation of D-arabitol using the mixed cultures of recombinant and original strains produced enhanced amount of xylitol without using any exogenous NADH supply. The concentration and yield of xylitol produced by the recombinant strains in this study are nicely comparable with those reported in the previous studies using the strains that required external NADH for converting D-arabitol. Therefore, strains with self-regenerating system of NADH could be considered as the promising biological agent for biotransformation of D-arabitol into xylitol.

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