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RESEARCH ARTICLE

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Coupled biosynthesis and esterification of 1,2,4-butanetriol to simplify its separation from fermentation broth

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1,2,4-Butanetriol (BT) is a valuable chemical with versatile applications in many fields and can be produced through biosynthetic pathways. As a trihydric alcohol, BT possesses good water solubility and is very difficult to separate from fermentation broth, which does complicate the production process and increase the cost. To develop a novel method for BT separation, a biosynthetic pathway for 1,2,4-butanetriol esters with poor water solubility was constructed. Wax ester synthase/acyl-coenzyme A: diacylglycerol acyltransferase (Atf) from *Acinetobacter baylyi*, *Mycobacterium smegmatis*, and *Escherichia coli* were screened, and the acyltransferase from *A. baylyi* (AtfA) was found to have higher capability. The BT producing strain with AtfA overexpression produced 49.5 mg/L BT oleate in flask cultivation. Through enhancement of acyl-CoA production by overexpression of the acyl-CoA synthetase gene *fadD* and deleting the acyl coenzyme A dehydrogenase gene *fadE*, the production was improved to 64.4 mg/L. Under fed-batch fermentation, the resulting strain produced up to 1.1 g/L BT oleate. This is the first time showed that engineered *E. coli* strains can successfully produce BT esters from xylose and free fatty acids.

KEYWORDS
1,2,4-butanetriol esters, acyltransferase, biosynthesis, separation, trihydric alcohol

1 | INTRODUCTION

1,2,4-Butanetriol (BT) is a valuable fine chemical that has versatile applications in many fields. For instance, BT can be used as raw material for making polyurethane foams with better elastic properties [1], and potential precursor for the synthesis of various pharmaceuticals [2]. BT is most widely applied to the production of 1,2,4-butanetriol trinitrate, an energetic plasticizer to replace nitroglycerin [3]. BT synthesis has aroused much interest as its potential applications.

Traditionally, BT is mainly manufactured through chemical routes using malate as the starting material [4,5]. However, the chemical synthesis is not competitive because of the harsh reaction conditions and poor selectivity. And $NaBH_4$ is required as the reducing agent resulting with plenty of borate salts generated [6]. In recent years, BT was successfully produced from cheap sugars through biological pathways [3,7,8], which possesses mild conditions and can reduce the environmental pollution compared to the traditional petrochemical routes. A patent claimed that 18 g/L BT could be achieved from xylose in a single strain with a yield of 0.55 mol/mol [9]. However, no reports were focused on its separation so far.

As a polyol, BT has three hydrophilic hydroxyl groups, which means good water solubility and very difficult to separate from water. Many methods were studied on the separation of polyol including evaporation, distillation, membrane filtration, ion exchange chromatography, and extraction. However, no single method proved simple, efficient, and low cost.

Abbreviations: Atf, wax ester synthase/acyl-coenzyme A: diacylglycerol acyltransferase; BT, 1,2,4-butanetriol; FFA, free fatty acid; IPTG, isopropyl-β-D-thiogalactopyranoside; TAG, triglyceride

Among these methods, liquid–liquid extraction can be easy to scale-up and has low energy consumption, but it is difficult to find an effective solvent for polyols [10]. The distillation method operates easily, but it consumes too much energy, especially the azeotropic phenomenon exists between water and polyols, which increases production cost. It is estimated that the separation of the polyols from fermentation broth makes about 50–70% of the total costs in their whole production process [11]. Salting-out is another alternative separation method, which was investigated on the recovery of 1,3-propanediol from fermentation broth [10]. Although the yield achieved more than 90%, much salt needed to add as salting-out agent.

Esters, usually derived from carboxylic acid and alcohol, confer poor water-solubility and can be easily separated from fermentation broth. Some previous studies have focused on the extraction and purification of lactic acid by coupling esterification and hydrolysis [12–14]. In these researches, lactate or ammonium lactate was esterified to methanol, ethanol, or butanol, following hydrolysis of the purified ester into lactate again to extract and purify. Both the yield and the purity reached about 90%, and no calcium salts generated. It seemed that the esterification and hydrolysis method was much more desirable compared with the conventional process, and actually inspired us to develop a similar method to recovery BT. The purpose of this work is to develop a biosynthetic pathway for 1,2,4-butanetriol esters production. We firstly introduced different wax ester synthase/acyl-coenzyme A: diacylglycerol acyltransferase to a BT producing strain to esterify BT. To improve the acyl-CoA as the ester precursor, the *fadE* gene encoding acyl coenzyme A dehydrogenase was deleted to block fatty acid β-oxidation, and *fadD* gene encoding acyl-CoA synthetase was overexpressed. The key enzymes *glpK*, *plsB*, and *pgpB* of triglyceride (TAG) biosynthesis were finally over-expressed to check whether it can enhance the BT esters production. The entire metabolic pathway used in this study is proposed in Fig. 1. The engineered strain cultivation was performed in fed-batch fermentation to evaluate the potential for large-scale production. This research will provide a new idea for the separation of polyol production from fermentation broth.

PRACTICAL APPLICATION

Due to good water solubility, polyhydric alcohols are very difficult to separate from fermentation broths. As a trihydric alcohol with versatile applications, 1,2,4-butanetriol can be produced by biological fermentation with good yield. However, no research was focused on its separation so far which hamper its industrialization promotion. In this study, BT esters with poor water-solubility was successfully produced by recombinant strains from xylose and oleate for the first time, and the fed-batch fermentation showed a promising prospect for the large-scale production. With further research, BT can be recovered more easily and cost-effective by centrifugation coupling with hydrolysis. The present study not only proved that BT esters can be produced through biosynthetic pathway but also provided an alternative method for trihydric alcohol separation from fermentation broth.

2 | MATERIALS AND METHODS

2.1 | Bacterial strains and plasmids construction

The strains and plasmids used in this study were presented in Table 1, and the primers used for plasmids construction and allele verification were listed in Supporting Information Table 1. *E. coli* DH5α purchased from Invitrogen was used for gene cloning plasmid maintenance, and *E. coli* BL21 star (DE3) was used as the host strain for gene expression. The genes *xylAB* deleted strain *E. coli* BL21 star (DE3) Δ*xylAB* was constructed in our previous study [15]. The chromosomal *fadE* gene encoding acyl coenzyme A dehydrogenase was knocked out using the λ-Red recombination method resulting strain *E. coli* BL21 star (DE3) Δ *xylAB* Δ *fadE*.

The plasmid pE-*mdlC-xylBC* and pA-*adhP-yjhG* were constructed in our previous study [8]. The wax ester synthase/acyl-coenzyme A: diacylglycerol acyltransferase from *Acinetobacter baylyi* (*atfA*), and *Mycobacterium smegmatis* (*atfM*) were codon optimized and chemically synthesized. The *atfE* gene amplified from *Escherichia coli*, *atfA*, and *atfM* were cloned into pA-*adhP-yjhG* between *EcoRI* and *SalI* sites to construct plasmids pA-*adhP-atfE-yjhG*, pA-*adhP-atfA-yjhG*, and pA-*adhP-atfM-yjhG*, respectively. All the *glpK*, *plsB*, *pgpB*, and *fadD* genes were PCR amplified from *E. coli* BL21. The *fadD* was cloned into pCOLADuet-1 vector between *AflII* and *KpnI* sites to generate pC-*fadD*, and the *glpK* was cloned into pC-*fadD* between *EcoRI* and *SacI* sites to create pC-*glpK-fadD*. The *plsB* was cloned into pC-*glpK-fadD* between *SacI* and *SalI* sites to obtain pC-*glpK-plsB-fadD*. Finally, the *pgpB* was amplified along with T7 promoter and then cloned into pC-*glpK-plsB-fadD* between *SalI* and *AflII* sites to get pC-*glpK-plsB-pgpB-fadD*. The resulting plasmid was verified by colony PCR, and nucleotide sequencing. And all the genes used in this study was listed in Supporting Information Table 2.

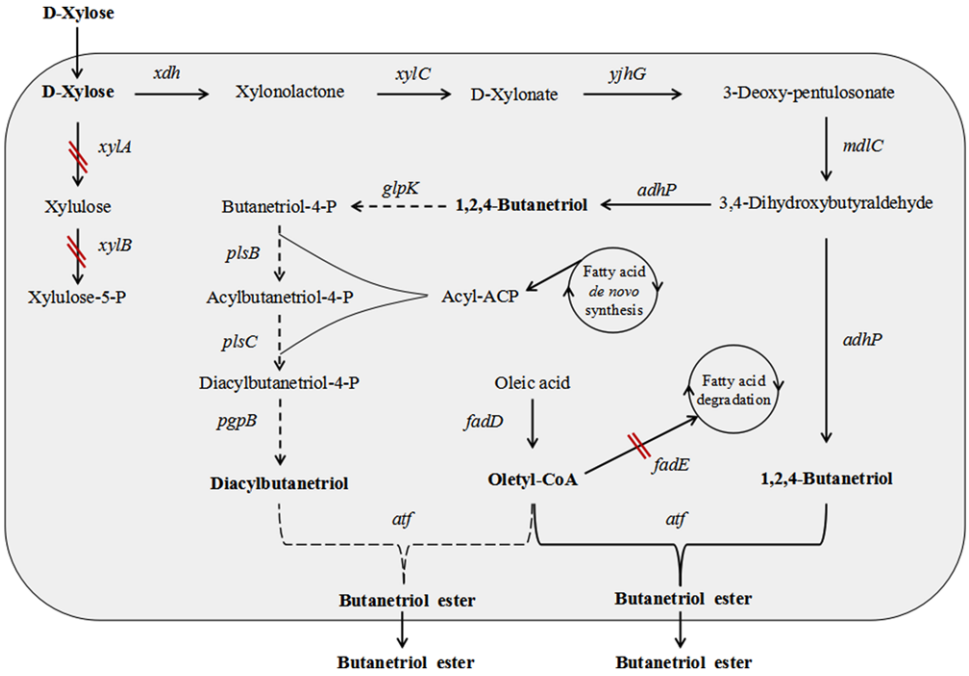

FIGURE 1 The metabolic pathway from xylose and oleate in engineered *E. coli* to BT oleate. The solid lines are the pathway used in this study for BT esters production, and the dashed lines are the presumed pathway. Genes: *xdh*, xylose dehydrogenase; *xylC*, xylonolactonase; *yjhG*, xylonate dehydratase; *mdlc*, 2-keto acid decarboxylase; *adhp*, aldehyde reductase; *xylA*, xylose isomerase; *xylB*, xylulose kinase; *glpK*, glycerol kinase; *plsB*, glycerol-3-phosphate acyltransferase; *plsC*, 1-acyl glycerol-3-phosphateacyltransferase; *pgpB*, diacylglycerol-3- phosphate phosphatase; *fadD*, acyl-CoA synthetase; *fadE*, coenzyme A dehydrogenase; atf, wax ester synthase/acyl-coenzyme A: diacylglycerol acyltransferase.

2.2 | Protein expression and gel electrophoresis analysis

To check the expression of the recombinant proteins, single colonies of *E. coli* BL21 (DE3) star harboring different recombinant plasmids were cultured in LB medium containing appropriate antibiotics at 37°C overnight and then diluted 1:100 into fresh LB medium and induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at an OD_{600} of 0.6~0.8. The cells were collected from 10 mL bacteria cultures 4h after induction and washed with phosphate buffer (pH 6.8). The washed cells were suspended in 1 mL buffer and subjected to ultrasonication. The cell lysates were centrifuged and the supernatant was analyzed by SDS-PAGE [16].

2.3 | Shake flask cultivation

To evaluate the ester production using different engineered strains, shake flask cultivation was carried out with 100 mL of liquid LB medium in 250 mL nonbaffled flasks with appropriate antibiotics. The strains were inoculated to the medium and incubated in an orbital incubation shaker at 37°C with 180 rpm. A 0.5 mM IPTG was added into the medium to induce the enzymes expression when the cells reached about 0.6 OD_{600}. 20 g/L xylose was added for BT production and 2 g/L sodium oleate was added as precursor for esters. After induction, the temperature was set at 30°C for further 24 h cultivation.

2.4 | Fed-batch fermentation

For large-scale production, fed-batch fermentation was carried out in a Biostat B plus MO5L bioreactor (Sartorius Stedim Biotech GmbH, Germany) containing 2 L growth medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, 5 g/L $K_2HPO_4{\cdot}3H_2O$, 0.12 g/L $MgSO_4$, 0.1 g/L of thiamine hydrochloride and 1 mL trace elements per liter [8]). Two hundred milliliters of overnight seed culture was inoculated into the fermentor to start the fermentation at 37°C. During the fermentation, sterilized air was supplied at 1 vvm and ammonia was added automatically to control the pH 7.0. The agitation speed was set at 400 rpm and then associated with the dissolved oxygen to maintain the concentration at 20% saturation. Fed-batch mode was commenced by feeding 5 × LB when the dissolved oxygen increased. When the cell density reached to an OD_{600} of 10, the recombinant proteins were induced by 0.5 mM IPTG, 20 g/L xylose and 10 g/L sodium oleate were added for BT esters production. 10 ml of the fermentation broth was withdrawn at intervals to determine cell density and products.

TABLE 1 Strains and plasmids used in this study

Strains or plasmids	Genotype/description	Source
Strains		
E. coli DH5α	F⁻ *recA endA1 Φ80dlacZΔM15 hsdR17*(r_K^- m_K^+) λ^-	Invitrogen
E. coli BL21 star (DE3)	F⁻ *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm rne131* (DE3)	Invitrogen
E. coli BL21 star (DE3) Δ *xylAB*	Knockout of *xylA* and *EcxylB* encoding xylose isomerase and xylulose kinase	Ref. 15
E. coli BL21 star (DE3) Δ *xylAB* Δ *fadE*	Knockout of *xylA*, *EcxylB* and *fadE* encoding xylose isomerase, xylulose kinase and acyl-CoA dehydrogenase	This study
Q2545	*E. coli* BL21 star (DE3)Δ *xylAB* / pACYCDuet-*adhP-atfA-yjhG* & pETDuet-*mdlC-xylBC*	This study
Q2546	*E. coli* BL21 star (DE3)Δ *xylAB* / pACYCDuet-*adhP-atfE-yjhG* & pETDuet -*mdlC-xylBC*	This study
Q2547	*E. coli* BL21 star (DE3)Δ *xylAB* / pACYCDuet-*adhP-atfM-yjhG* & pETDuet -*mdlC-xylBC*	This study
Q2714	*E. coli* BL21 star (DE3) Δ *xylAB* Δ *fadE* / pET-*mdlC-xylBC* & pACYCDuet-*adhP-atfA-yjhG* & pCOLA- *fadD*	This study
Q3016	*E. coli* BL21 star (DE3) Δ *xylAB* Δ *fadE* / pET-*mdlC-xylBC* & pACYCDuet-*adhP-atfA-yjhG* & pCOLA-*glpK-plsB -pgpB-fadD*	This study
Plasmids		
pCOLADuet-1	*Kan*^{r} *oriColA lacI*^{q} *T7*$_p$	Novagen
puC57-*atfM*	puC57 harboring *M. smegmatis atf*	Genewiz
puC57-*atfA*	puC57 harboring *A. baylyi atf*	Genewiz
pE-*mdlC-xylBC*	pETDuet-1 harboring *P. putida mdlC* and *C. crescentus CCxylB* and *xylC*	Ref. 8
pA-*adhP-yjhG*	pACYCDuet-1 harboring *E. coli yjhG* and *adhP*	Ref. 8
pA-*adhP-atfA-yjhG*	pACYCDuet-1 harboring *E. coli yjhG*, *adhP* and *A. baylyi atf*	This study
pA-*adhP-atfE-yjhG*	pACYCDuet-1 harboring *E. coli yjhG*, *adhP* and *atf*	This study
pA-*adhP-atfM-yjhG*	pACYCDuet-1 harboring *E. coli yjhG*, *adhP* and *M. smegmatis atf*	This study
pC-*fadD*	pCOLADuet-1 harboring *E. coli fabD*	This study
pC-*glpK-plsB-pgpB-fadD*	pCOLADuet-1 harboring *E. coli glpK, plsB, pgpB, and fabD*	This study

2.5 | Analytic methods

BT esters were extracted by adding 50 mL of organic solvent containing chloroform and methanol (the ratio is 2:1 by v/v) to an equal volume of whole culture with 0.1 mg nonadecanoic acid methyl ester added as an internal standard and vortexing for 30 seconds. After phase separation, the organic phase was evaporated by a rotary evaporator and redissolved in 2 mL of organic solvent containing chloroform and methanol.

Samples were analyzed by LC-MS. UHPLC analysis was carried out using an Ultimate 3000 UHPLC (Thermo, USA) with a Thermo Acclaim RSLC C18 column (2.1 mm × 100 mm, 2.2 μm) with Thermo online UHPLC filter (2.1 mm, 0.2 μm) used for the chromatographic separation. The mobile phase A consisted of 0.1% of formic acid and 2 mM ammonium formate in 2-propanol/water (98: 2), and the mobile phase B was composed of methanol/water with 0.1% formic acid and 2 mM ammonium formate (98: 2). The elution gradient was started at 100% B for the first 3 min with flow rate of 0.2 mL/min, stepping to 75% B at 3.1 min, linearly ramped to 50% B at 20 min, linearly ramped to 17% B at 36 min, stepping to 0% B and 0.25 mL/min at 36.2 min, holding at 0% B until 43 min, and returning to 100% B with the increased flow rate of 0.2 mL/min at 43.1 min, holding these conditions at 50 min and stopping the controller. The injection volume was 2 μL and the column temperature was 30°C.

A Compact Q-TOF mass spectrometry (Bruker Daltonics, Billerica, USA) with an ESI source in positive ion mode with HyStar 3.2 software was used to link the LC and the MS, using the following operation parameters: capillary voltage 4500 V, dry temperature 200°C, nebulizing gas of 1.5 bar, drying gas (N_2, purity 99.999%) flowing of 5.5 L/min. High resolution MS and MS/MS spectra were acquired in the range 50–1300 *m/z*. The collision gas was high purity nitrogen (purity 99.99%). The data were collected by auto MS/MS acquisition with a MS scan rate of 1 spectra/s and MS/MS scan rate of three precursor was acquired per cycle, active exclusion after 3 spectra and 1.0 min. OTOF Control software was used to carry out mass spectrometer control and data acquisition and Compass Data Analysis soft was applied for data analysis.

3 | RESULTS AND DISCUSSION

3.1 | Product verification and Atf screening

A novel bifunctional wax ester synthase/acyl-CoA: diacylglycerol acyltransferase was found in *Acinetobacter baylyi* ADP1 exhibiting acyl-CoA: fatty alcohol acyltransferase as well as acyl-CoA: diacylglycerol acyltransferase activity, which can utilize C_{12}-C_{20} carbon length fatty acids and fatty alcohols [17]. In another report, the AtfA was used for ethyl oleate production in metabolically engineered *Escherichia coli*, which means short chain alcohols can also be used as substrate for AtfA [18]. Based on its broad substrate range, we speculated that BT can be esterified with fatty acids by AtfA.

In order to obtain BT esters, the AtfA was introduced into a BT producing strain constructed in our previous study [8], the resulting strain Q2545 was cultured in LB medium with xylose and sodium oleate. After fermentation, the extracted production was analyzed by LC-MS. As the skeletal structure, the BT molecule can be esterified with 1, 2, or 3 oleate molecules. The proposed formulas and ions of the mass spectra for the identified BT esters are shown in Table 2. As an example, when one BT molecule was esterified with two oleate molecule, the ions at 635.6254 *m/z* ($[M + H]^+$), 652.6258 *m/z* ($[M + NH_4]^+$), and 657.6095 *m/z* ($[M + Na]^+$) can be observed in the mass spectrum. As Supporting Information Figure 1 demonstrated, except that the BT esterification products with three oleate molecules only obtained the ion of 916.8315 *m/z* ($[M + NH_4]^+$), all the other specific ions appeared. The reason for this phenomenon might be the fairly low amount of the product. For the MS^2 spectrum, the characteristic spectra of 265 *m/z* ($C_{18}H_{34}O_2$-OH) were observed in all the three different scenarios. The results described above clearly proved that BT oleate biosynthesis is feasible in recombinant *E. coli* with heterologous expression of AtfA.

Until now, enzymes similar to AtfA have been identified in several different types of bacteria [19]. To enhance the production, the key enzyme was screened. Two more genes encoding Atf from *Mycobacterium smegmatis* (*atfM*, GeneID: 4537099) and *E. coli* (*atfE*, GeneID: 947324) were cloned and used for BT esters production. After 24 h cultivation postincubation, the strain Q2545 carrying *atfA* produced 49.5 mg/L BT oleate (Figure 2). Atf in *M. smegmatis* mc^2 *155* was up to 41% amino acid identity to AtfA, and the activity of AtfM was

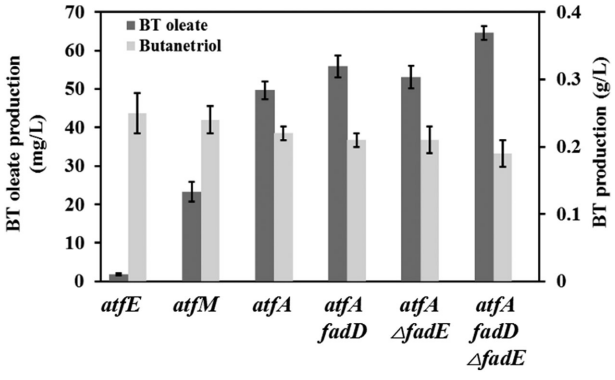

FIGURE 2 Comparison of BT esters production in flask cultivation by different engineered strains. Data were obtained after each strain was induced for 24 h in liquid LB medium. All the experiments were performed in triplicate.

TABLE 2 Proposed fragmentations of the tandem mass spectra for the identified BT esters

Skeletal structure	Esterification with oleate	MS^1 formula, and ion (*m/z*)
1,2,4-butanetriol, $C_4H_{10}O_3$	1 oleic acid, $C_{22}H_{42}O_4$	$[M + H]^+$, 371.35
		$[M + NH_4]^+$, 388.38
		$[M + Na]^+$, 393.34
	2 oleic acid, $C_{40}H_{74}O_5$	$[M + H]^+$, 635.63
		$[M + NH_4]^+$, 652.65
		$[M + Na]^+$, 657.61
	3 oleic acid, $C_{58}H_{106}O_6$	$[M + H]^+$, 899.81
		$[M + NH_4]^+$, 916.83
		$[M + Na]^+$, 921.78

106.54 pmol/(mg min) that is higher than AtfA with 90.37 pmol/(mg min) [17]. However, the strain carrying *atfM* just produced 23.3 mg/L BT oleate that is less than half of that of strain Q2545. It was speculated that AtfM has higher substrate specificity than AtfA and the underlying reason need revealed in future study. A predicted acyltransferase from *E. coli* (*atfE*) was also cloned for BT oleate. Due to AtfE prefer short-chain acyl-CoA [20], the production in the present study reached just 1.8 mg/L.

3.2 | Expression and identification of recombinant enzymes

To make sure all the genes used in this study were successfully expressed in the host strain, the recombinant plasmids were transformed into *E. coli* BL21 star (DE3) and the expression level of the recombinant proteins were verified by SDS-PAGE. All the strains were cultured in liquid LB medium and 0.5 mM IPTG were added to induce the expression of the recombinant proteins. The bands with the expected size of the recombinant proteins from crude extracts of the recombinant strains appeared obviously in comparison with the control strain containing empty vector. As shown in Supporting Information Figure 2, the various recombinant strains carrying the MdlC, XylB, XylC, AdhP, and YjhG enzymes that constitute the entire BT metabolic pathway from xylose revealed the corresponding bands with molecular weights of 56.4 kDa, 26.6 kDa, 31.6 kDa, 35.4 kDa, and 70 kDa. And the Atf from *A. baylyi* (50.5 kDa for AtfA) was also successfully expressed, presenting the expected protein band. Finally, all the enzymes for BT ester production were properly expressed in the resulting strain.

3.3 | Effect of *fadD* overexpression and *fadE* deletion

A sufficient amount of precursors is necessary to the efficient synthesis of the final products. The amount of acyl-CoA can be increased by activating the fatty acid de novo synthesis or by blocking their degradation via β-oxidation. In *E. coli*, free fatty acids (FFA) can be esterified to acyl-CoAs by the acyl-CoA synthetase FadD [21]. There are few studies describing significant triacylglycerols (TAG) synthesis in *E. coli* by overexpressing *fadD* and *atfA* [22]. As an alternative strategy for enhanced yields of FFA, *fadE* has been deleted in several studies to interrupt fatty acid degradation [23,24]. To improve the BT oleate production, overexpression of *fadD* or deleting of *fadE* was performed, *fadD* was cloned into vector pCOLADuet-1 between sites *BglII* and *KpnI*, and the *fadE* gene was deleted using the λ-Red recombination method. Under the same cultivation conditions, the production of BT oleate was a little higher than the strain of Q2545, with *fadD* overexpressed or *fadE* deleted independently (Figure 2). And the resulting strain Q2714 obtained 64.4 mg/L BT oleate that is 1.3-fold higher than the strain Q2545 (Figure 2).

The acyltransferase played an important role in TAG biosynthesis pathway. There are at least five steps for converting glycerol into TAG. Glycerol was firstly converted to glycerol-3-phosphate by glycerol kinase GlpK, then converted into diacylglycerol step by step by glycerol-3-phosphate acyltransferase PlsB, 1-acyl glycerol-3-phosphateacyltransferase PlsC, and diacylglycerol-3-phosphate phosphatase PgpB, and finally esterified by acyltransferase with acyl-CoA [25]. BT (1,2,4-butanetriol) has the similar structure as that of glycerol (propanetriol), which enlightened us to enhance the BT oleate production using the TAG synthesis pathway. So, the genes *glpK*, *plsB*, and *pgpB* were cloned and overexpressed to verify the influence. However, the yield of the product was not further improved by the resulting strain Q3016 (63.2 mg/L). Moreover, more complex mass spectrum was observed which means some other products produced along with the TAG biosynthesis pathway, and further study was needed in the future to confirm this speculation.

3.4 | Fed-batch fermentation

In order to test the potentiality of the recombinant strain for larger scale production of BT oleate, fed-batch fermentation

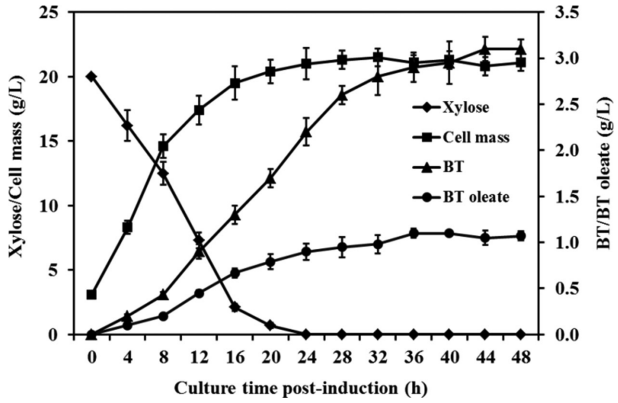

FIGURE 3 The time profiles of cell growth, residual xylose, BT, and BT oleate concentrations in culture broth during the aerobic fed-batch fermentation of the engineered strain Q2714. Cultures were performed in a 5-L laboratory bioreactor.

was established in a 5 L-scale laboratory fermenter using Q2714. The concentrated LB was used for cell growth, 0.5 mM IPTG was added to induce the recombinant proteins when the cell density reached to about 10 OD_{600}, and 20 g/L xylose and 10 g/L sodium oleate were used for BT oleate biosynthesis. Cell growth, residual xylose and product accumulation were monitored over the course of the fermentation. As shown in Figure 3, the bacteria grew rapidly and the cell mass reached to about 21.5 g/L at 32 h postinduction. A 20 g/L xylose for BT biosynthesis was almost exhausted within 24 h. BT oleate accumulated after induction in the culture broth and obtained 1.1 g/L at 36 h postinduction and then gradually to a stable value, corresponding to a productivity of 30.6 mg/L/h. An amount of BT was still detected in the broth during the fermentation, which means that it can't be converted into esters effectively. It is necessary to screen high-efficiency acyltransferase or improve the catalytic activity with enzyme engineering. Also, incorporate the *atf* gene in the genome, optimize the gene expression through promoter selection, and other methods should be considered in future study. As the cultivation conditions might affect the product synthesis and the conversion efficiency, it is worthy to optimize the fermentation mode, aeration condition, and substrate addition strategy in further research. The BT production can be recovered by centrifugation coupling with hydrolysis reaction. Compared to the traditional distillation, evaporation and other methods, the procedure is simplified and consumes lower energy, which can reduce the process cost. It is believed that this research will provide a new idea for simplifying the separation and purification process and reduce the cost of BT production. Considering potential problems, maybe it still need to combine the present method with other technologies to establish a more energy saving and efficient method for BT separation.

4 | CONCLUDING REMARKS

1,2,4-Butanetriol was successfully esterified with oleate in recombinant *E.coli* strains with wax ester synthase/acyl-coenzyme A: diacylglycerol acyltransferase in this study. When *atf* from *Acinetobacter baylyi* and *fadD* from *E. coli* were overexpressed in the BT producing strain with *fadE* gene deletion, 64.4 mg/L BT oleate was accumulated in flask cultures, and the yield of the product reached to 1.1 g/L in fed-batch fermentation. This is the first time to achieve BT esters through biosynthetic pathway.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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