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Engineering microaerobic metabolism of *E. coli* for 1,2-propanediol production

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Abstract Establishment of novel metabolic pathways for biosynthesis of chemicals, fuels and pharmaceuticals has been demonstrated in *Escherichia coli* due to its ease of genetic manipulation and adaptability to varying oxygen levels. *E. coli* growing under microaerobic condition is known to exhibit features of both aerobic and anaerobic metabolism. In this work, we attempt to engineer this metabolism for production of 1,2-propanediol. We first redirect the carbon flux by disrupting carbon-competing pathways to increase the production of 1,2-propanediol microaerobically from 0.25 to 0.85 g/L. We then disrupt the first committed step of *E. coli*'s ubiquinone biosynthesis pathway (*ubiC*) to prevent the oxidation of NADH in microaerobic conditions. Coupling this strategy with carbon flux redirection leads to enhanced production of 1,2-propanediol at 1.2 g/L. This work demonstrates the production of non-native reduced chemicals in *E. coli* by engineering its microaerobic metabolism.

Keywords 1,2-Propanediol · Microaerobic · Ubiquinone · *Escherichia coli*

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Introduction

Over the last decade, the biosynthesis of various high-value and commodity chemicals has been established by engineering *Escherichia coli* [1, 4, 9, 12, 14, 15, 18, 21, 26, 32]. As a facultative anaerobe, *E. coli*'s metabolism provides the freedom to explore the production of target chemicals at varying oxygen levels. In this light, the production of target chemicals has been achieved via adapting process parameters (oxygen availability) favoring metabolic logic. Under aerobic conditions, generation of oxidized products is favored, ATP production is driven, and a significant amount of carbon is lost toward generation of biomass and CO_2. Anaerobically, the production of reduced chemicals is favored due to the prevention of NADH oxidation, while limiting ATP generation and carbon loss toward biomass [33]. To take advantage of both aerobic and anaerobic metabolic profiles, the use of microaerobic condition (limited oxygen supply) has been demonstrated for biosynthesis of target chemicals [28, 33]. However, the production of reduced chemicals in the presence of oxygen is challenging as most of the intracellular NADH is oxidized, thereby undermining the synthetic pathway.

A strategy used to promote the production of reduced products under microaerobic or anaerobic conditions involves the expression of NAD^+-dependent *Candida boidinii* formate dehydrogenase. It has been shown that this results in intracellular NADH regeneration favoring reduced chemicals formation [6]. However, this approach requires the expression of a heterologous enzyme and additional supply of formate to the culture, thereby increasing the complexity of the process. Another strategy to favor native reduced chemicals production aerobically has been reported recently via the disruption of *E. coli*'s electron transport chain [23, 24, 27]. It was reported that the

disruption of the first two committed steps (*ubiCA*) leading to ubiquinone biosynthesis resulted in increasing lactate production under aerobic conditions, however, also causing a significant reduction in cell growth [33]. The addition of 1 μM of an analog of ubiquinone—coenzyme Q1 caused a shift in the metabolism, which nearly eliminated lactate generation in *ldhA ubiCA* mutant strain, while promoting the production of ethanol and acetate. Interestingly, increasing coenzyme Q1 supplementation beyond 5 μM did not result in enhancing reduced chemicals production or improving cell growth. It was speculated that the low solubility of coenzyme Q1 may limit its uptake by *E. coli*. Another speculation was that the efficiency of coenzyme Q1 was much lower than that of ubiquinone [33].

Although a strategy to conserve NADH was described by engineering the electron transport chain, significant drawbacks may limit its use for large-scale manufacture of chemicals. For instance, (a) the supplementation of coenzyme Q1 is expensive, (b) coenzyme Q1 is not soluble in the media at high concentrations, and (c) reduction in cell growth may reduce productivity. A solution to this can be to down-regulate the metabolic pathway to ubiquinone biosynthesis, instead of its complete disruption [33]. It has been reported previously that the disruption of only the first committed step to ubiquinone biosynthesis (*ubiC*) in *E. coli* leads to a significant reduction in ubiquinone biosynthesis, but does not completely eliminate it [20]. This strategy may conserve NADH by preventing its oxidation to a large extent, thereby promoting reduced chemicals formation without significantly hampering cell growth.

In this work, we utilize this strategy to demonstrate the microaerobic production of a non-native reduced high-value bulk chemical—1,2-propanediol. 1,2-Propanediol has an annual market of over 1 billion pounds, and is manufactured via chemical methods from propylene via hydration, or via high-pressure and high-temperature hydrogenolysis of sugars; however, these methods are expensive and harmful to the environment [2]. An additional route to the manufacture of 1,2-propanediol via fermentation is also known, via utilization of expensive sugars like L-rhamnose (>$300/kg), making it not feasible for large-scale manufacture [2]. Hence, the manufacture of 1,2-propanediol biologically via relatively inexpensive carbon sources holds great commercial value. Here, first the microaerobic production of 1,2-propanediol in wild-type *E. coli* is pursued from glucose. Next, by engineering the strain to effectively redirect the carbon flux into 1,2-propanediol pathway, its production was enhanced from 0.25 to 0.85 g/L. To test our hypothesis, we further engineer the strain by disrupting chorismate pyruvate lyase (*ubiC*) activity. In doing so, a further increase in 1,2-propanediol production to 1.2 g/L is reported. This work demonstrates a new approach to conserve intracellular cofactors without requiring the additional supply of expensive media components, making it an employable strategy for large-scale production of reduced chemicals.

Materials and methods

Chemicals, bacterial strains and plasmids

Chemical standards of methylglyoxal and 1,2-propanediol were purchased from Sigma Aldrich (St. Louis, MO); hydroxyacetone standard was purchased from Acros Organics (New Jersey, USA). GoTaq DNA polymerase was procured from Promega (Madison, WI); Phusion high fidelity DNA polymerase, quick DNA ligase and restriction enzymes were procured from New England Biolabs (Beverly, MA).

Microbial biosynthesis studies were carried out using *E. coli* BW25113 and its knock-out derivatives were obtained from *E. coli* Genetic Resource Center (as listed in Table 1) [5]. To construct knock-out strains with more than one gene disruption, P1 transduction was used as described previously [29]. To facilitate the loss of kanamycin resistance from engineered strains, plasmid pCP20 was used [11]. All engineered strains were verified via colony PCR. *E. coli* XL1-Blue (Stratagene, CA) was used as the cloning host. The cloning plasmid used was pZE12-luc [22]. The plasmids pRJ11 and pRJ70 were constructed as described previously. To create pRJ11, the genes *ydjG* from *E. coli*, *budC* from *Klebsiella pneumoniae* and *mgsA* from *Bacillus subtilis* were inserted into the backbone of pZE12-luc [17]. The genes *mgsA* from *B. subtilis*, *gldA* and *fucO* from *E. coli* were inserted into the backbone of pZE12-luc to create pRJ70 [16].

Culture medium and shake flask studies

The modified M9 media consisted of (per liter): 12.8 g Na_2HPO_4 $7H_2O$, 3 g KH_2PO_4, 1 g NH_4Cl, 0.5 g NaCl, 0.5 mM $MgSO_4$, 0.05 mM $CaCl_2$, 20 g glucose and 5 g yeast extract [17]. The biosynthesis of 1,2-propanediol was carried out with modifications to the method described previously [17]. Seed cultures (3 mL) were prepared by growing the transformants overnight at 37 °C, 250 rpm in LB. Shake flask studies for biosynthesis were begun with the addition of 200 μl of seed cultures into 125 mL screw cap bottles containing 20 mL modified M9 media with appropriate antibiotics. The cultures were then grown at 37 °C for 4 h, at 250 rpm. At this time, protein expression was induced, with the addition of 0.1 mM IPTG to the cultures. Following this, the cultures were then grown at 30 °C for another 92 h at 250 rpm. The screw cap bottles were tightened to prevent exposure to oxygen, thereby making

Table 1 List of strains and plasmids used in this study

Strain	Genotype	Reference
E. coli BW25113	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ⁻, rph-1, Δ(rhaD-rhaB)568, hsdR514*	Yale CGSC
E. coli XL-1 Blue	*recA1 endA1gyrA96thi-1hsdR17supE44relA1lac [F' proAB lacIqZDM15Tn10 (TetR)]*	Stratagene
JW2293-1	BW25113 Δ*ackA::kan*	Yale CGSC
JW0855-1	BW25113 Δ*poxB::kan*	Yale CGSC
JW3890-2	BW25113 Δ*tpiA::kan*	Yale CGSC
JW1841-1	BW25113 Δ*zwf::kan*	Yale CGSC
JW5713-1	BW25113 Δ*ubiC::kan*	Yale CGSC
BW25113 Δ*ackA* Δ*poxB*	BW25113 with *ackA* and *poxB* disruptions	[28]
RJ29	BW25113 with *zwf* and *tpiA* disruptions	This study
RJ40	BW25113 with *zwf*, *tpiA*, *ackA* and *poxB* disruptions	This study
RJ147	BW25113 with *zwf*, *tpiA*, *ackA*, *poxB* and *ubiC* disruptions	This study

Plasmid	Description	Reference
pZE12- luc	pLlacO1; luc; *ColE1 ori*; Amp^R	[22]
pRJ11	*ydjG* from *E. coli*, *budC* from *K. pneumoniae* and *mgsA* from *B. subtilis* cloned in pZE12-luc	[17]
pRJ70	*mgsA* from *B. subtilis*, *gldA* from *E. coli* and *fucO* from *E. coli* cloned into pZE12-luc	[16]

the culture oxygen limited (microaerobic condition) as described previously [28]. 1 mL samples were collected for analysis of cell growth and products. All experiments were performed in parallel with 3 replicates.

Analytical procedures

Optical density was measured at 600 nm using a Genesys 10S UV–Vis Spectrophotometer (Thermo Scientific, Waltham, MA). For product quantification and separation, 1 mL samples were collected and prepared prior to analysis as described previously [17]. For quantification of cellular metabolites HPLC–RID (Shimadzu) with Coregel-64H column (Transgenomic) was used. The eluent used was 4 mN H_2SO_4 with a flow rate of 0.6 mL/min. The oven temperature was set at 60 °C for separation of native organic acids and 1,2-propanediol [13, 16]. The quantification of oxygen level in shake flasks was not done due to technical difficulties.

Results and discussion

1,2-Propanediol biosynthesis

The biological production of 1,2-propanediol from *E. coli* and *Saccharomyces cerevisiae* has been extensively explored recently due to its high demand as a bulk chemical [2, 3, 7, 10, 16, 17, 19, 25]. In our initial work, a dual biochemical route to 1,2-propanediol was established under anaerobic conditions. This included the expression of methylglyoxal synthase (*mgsA*), which catalyzes the formation of methylglyoxal from DHAP; following which either hydroxyacetone or lactaldehyde was formed by the action of methylglyoxal reductase (*ydjG*) or secondary alcohol dehydrogenase (*budC*), respectively. The generation of 1,2-propanediol from hydroxyacetone or lactaldehyde was achieved by the action of secondary (*budC*) or primary (*fucO*) alcohol dehydrogenase, respectively [17]. Building on this work, a more efficient solitary biochemical route to 1,2-propanediol was established via lactaldehyde anaerobically, with the expression of optimal minimal set of enzymes (*mgsA*, *gldA*, *fucO*) [16] (Fig. 1).

In this work, the efficiency of the dual route was first evaluated as compared to the solitary route for microaerobic production of 1,2-propanediol from glucose. Wild-type *E. coli* BW25113 was transformed with either plasmid pRJ11 or pRJ70 carrying the genes expressing the enzymes involved in the dual or solitary routes, respectively. Shake flask studies (20 mL) were carried out using modified M9 media as described in "Materials and methods". As seen from Fig. 2, after 48 h of microaerobic studies, the strain carrying pRJ11 produced 0.15 g/L of 1,2-propanediol, while the strain carrying pRJ70 produced 0.22 g/L of 1,2-propanediol. This observation was consistent with our previous work, where the expression of optimal minimal set of enzymes via plasmid pRJ70 enhanced the anaerobic production of 1,2-propanediol. No intermediates (methylglyoxal, hydroxyacetone, lactaldehyde) were detected in both cases. The dominant byproduct produced after 48 h was found to be acetate, produced at 3.42 g/L. Other

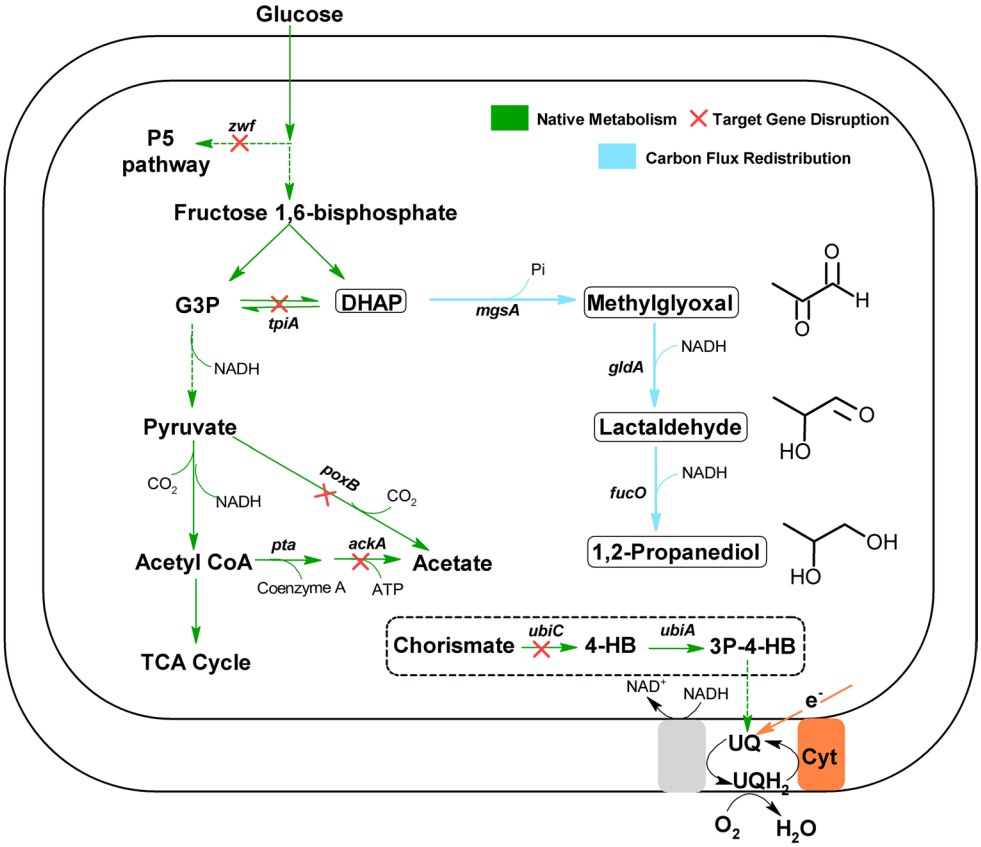

Fig. 1 Established 1,2-propanediol production pathway and native ubiquinone biosynthesis pathway in *E. coli*. *P5* pentose phosphate pathway, *G3P* glyceraldehyde 3-phosphate, *DHAP* dihydroxyacetone phosphate, *4-HB* 4-hydroxybenzoate, *3-P-4-HB* 3-octaprenyl-4-hydroxybenzoate, *UQ/UQH*$_2$ ubiquinones, *Cyt* cytochrome

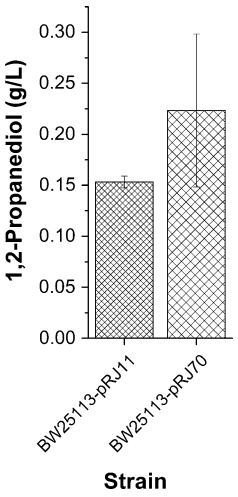

Fig. 2 1,2-Propanediol production from wild-type *E. coli* BW25113 carrying different plasmids after 48 h of microaerobic shake flask studies

byproducts such as lactate, ethanol, formate, pyruvate and succinate were not accumulated at significant amounts (<0.2 g/L). Hence, we proceeded to study the effect of disrupting the acetate-producing pathways.

Disruption of acetate-producing pathways

We have previously demonstrated the beneficial effect of reducing acetate accumulation for production of (*R,R*)-2,3-butanediol under microaerobic conditions [28]. Here, we reasoned that by disrupting the acetate-producing pathways, carbon may be conserved for use in the 1,2-propanediol pathway. In addition, the accumulation of acetate at harmful levels may be detrimental to cell growth and protein expression during scale-up studies [30, 31]; hence, *E. coli* strain BW25113 was engineered by disrupting *ackA* and *poxB* genes. Plasmid pRJ70 was then transferred into this strain and shake flask studies were carried out for 96 h. As seen from Fig. 3a, the cell growth of this strain was higher than the wild-type strain (OD_{600} = 3.6), achieving a final optical density of 5.7 at the end of 96 h. Surprisingly, the production of 1,2-propanediol was reduced from 0.25 to 0.17 g/L after 96 h of shake flask studies (Fig. 3b). As expected, this strain accumulated lower amount of acetate (2.97 g/L) at the end of 96 h, as compared to 4 g/L from the wild-type strain (Fig. 3c). Consistent with other studies, we observed that the disruption of acetate-producing pathways (*ackA*/*poxB*) does not completely eliminate acetate production [8, 28]. Interestingly, it was observed that this strain

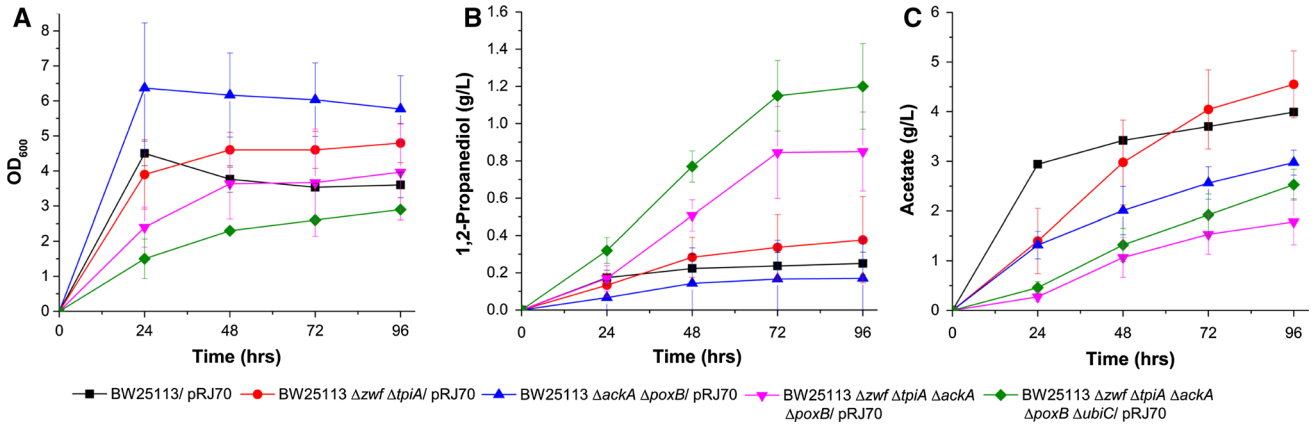

Fig. 3 Microaerobic studies for 1,2-propanediol production using different *E. coli* strains carrying plasmid pRJ70. **a** Growth curve of different *E. coli* strains. **b** 1,2-Propanediol production over 96 h of shake flask studies. **c** Acetate produced over 96 h of shake flask studies

accumulated pyruvate at nearly 1 g/L. This result indicated that just the disruption of major byproduct pathway (acetate) alone was not sufficient to improve 1,2-propanediol production. Furthermore, this suggested that it may be necessary to disrupt the major carbon-competing pathways (glucose 6-phosphate dehydrogenase and triose phosphate isomerase) to improve the production of 1,2-propanediol (Fig. 1) [16].

Disruption of carbon-competing pathways

As seen from Fig. 1, glucose 6-phosphate dehydrogenase (*zwf*) and triose phosphate isomerase (*tpiA*) direct the carbon into native metabolism. Hence, we speculated that the disruption of *zwf* and *tpiA* genes may result in increasing 1,2-propanediol titer. To test this hypothesis, *E. coli* strain RJ29 was constructed with *zwf* and *tpiA* gene disruptions and transformed with plasmid pRJ70, following which shake flask studies were carried out.

As seen from Fig. 3b, 1,2-propanediol production was increased to 0.38 g/L at the end of 96 h, as compared to 0.25 g/L or 0.17 g/L from wild-type or acetate pathways disrupted strains. Interestingly, the disruption of *zwf* and *tpiA* did not reduce the cell growth of this strain, achieving a final optical density of 4.8 after 96 h. Contrary to our expectation, this strain produced the maximum amount of acetate at 4.55 g/L. No pyruvate accumulation was observed from this strain. Since this strain resulted in increased 1,2-propanediol production, we hypothesized that the combined effect of disrupting *zwf*, *tpiA* and acetate consuming pathways will further enhance 1,2-propanediol production.

To test this hypothesis, *E. coli* strain RJ40 was constructed with *zwf*, *tpiA*, *ackA* and *poxB* gene disruptions and transformed with plasmid pRJ70. After 96 h of shake flask studies, it was seen that 1,2-propanediol production was increased to 0.85 g/L (Fig. 3b), representing a 3.4 fold increase as compared to the wild-type strain. As expected this strain reduced acetate generation to 1.78 g/L, representing a decrease of 2.5 fold as compared to strain RJ29 with the acetate pathways (4.55 g/L). Thus, the combined effect of disrupting the major carbon-competing pathways (*zwf*, *tpiA*) and the major byproduct pathways (*ackA*, *poxB*) resulted in enhancing 1,2-propanediol titer from 0.25 to 0.85 g/L.

Disruption of chorismate pyruvate lyase activity

Although 1,2-propanediol titer was increased by preventing loss of carbon, we speculated that its production was limited by NADH availability. As seen from Fig. 1, the production of 1,2-propanediol requires the consumption of 2 NADH molecules. It is understood that in the presence of oxygen cellular metabolism favors ATP generation, which involves the utilization of NADH molecules. To conserve intracellular NADH for reduced chemicals production, we investigated the effect of disrupting *E. coli*'s electron transport chain. It has been previously shown that the complete disruption of the ubiquinone biosynthesis pathway (*ubiCA*-) results in the reduction of cell growth, requires the supplementation of ubiquinone analogs for improving cell growth and reduced product formation [33]. In another study, it has been determined that the disruption of *ubiC* alone reduces intracellular ubiquinone levels, but does not eliminate it [20]. It was determined that even in the absence of 4-hydroxybenzoate, the *E. coli ubiC* mutant produced ubiquinone at about 20 nmol/g cell [20]. In this work, we hypothesized that the disruption of just the first committed step towards ubiquinone biosynthesis—chorismate pyruvate lyase (*ubiC*) will help conserve NADH by reducing

the amount of ubiquinone produced. We speculated that disrupting *ubiC* in addition to *zwf*, *tpiA*, *ackA* and *poxB* will result in further enhancing 1,2-propanediol production.

To test this hypothesis, *E. coli* strain RJ147 was constructed with *zwf*, *tpiA*, *ackA, poxB* and *ubiC* gene disruptions. This strain was transformed with plasmid pRJ70 and shake flask studies were carried out. As seen from Fig. 3b, the combined effect of carbon flux redirection and disruption of ubiquinone biosynthesis pathway (*ubiC*) resulted in increasing 1,2-propanediol production by nearly fivefold as compared to the wild-type strain from 0.25 to 1.2 g/L. The byproducts produced were acetate at 2.53 g/L, ethanol at 0.34 g/L, formate at 0.2 g/L. Pyruvate, succinate and lactate were produced at low amounts (<0.1 g/L). This strain achieved a final optical density of 2.7 as compared to 3.6 achieved by wild-type strain. Hence, this strategy improved reduced product formation, without dramatic reduction in cell growth and without the additional supply of expensive media components.

Furthermore, the effect of disrupting *ubiC* alone on 1,2-propanediol production was studied (data not provided). *E. coli* strain JW5713-1 with *ubiC* disruption was transformed with plasmid pRJ70 and shake flask studies were carried out as described previously. It was observed that after 96 h of shake flask studies, 1,2-propanediol production was only marginally increased as compared to the wild-type strain from 0.25 to 0.29 g/L. Therefore, we concluded that the combined effect of *ubiC* disruption along with carbon flux redirection was necessary to greatly improve the reduced product formation.

Conclusion

In this work, a new approach to enhance the production of reduced products microaerobically is established. We demonstrated the production of a high-value reduced bulk chemical—1,2-propanediol in *E. coli*. To achieve this, first the major competing pathways leading to acetate production were disrupted. However, this resulted in decreasing 1,2-propanediol titer to 0.17 g/L (Fig. 3b) and accumulated pyruvate at nearly 1 g/L. The major carbon-competing pathways were then disrupted in addition to the acetate-producing pathways. This strategy resulted in increasing 1,2-propanediol titer to 0.85 g/L. To conserve more NADH, we then disrupted the ubiquinone biosynthesis pathway (*ubiC*) in addition to the competing pathways (*zwf*, *tpiA*, *ackA* and *poxB*). The combined effect of these strategies improved reduced product formation, resulting in a 1,2-propanediol titer of 1.2 g/L after 96 h of shake flask studies. This approach has the advantage of not requiring supplementation of expensive media components, making it a more suitable strategy for large-scale production of reduced chemicals microaerobically as compared to previous efforts.

Conflict of interest The authors declare no competing interests.

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