



Application of a dissolved oxygen control strategy to increase the expression of *Streptococcus suis* glutamate dehydrogenase in *Escherichia coli*

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Abstract

The accumulation of acetate in *Escherichia coli* inhibits cell growth and desired protein synthesis, and cell density and protein expression are increased by reduction of acetate excretion. Dissolved oxygen (DO) is an important parameter for acetate synthesis, and the accumulation of acetate is inversely correlated to DO level. In this study, the effect of DO levels on glutamate dehydrogenase (GDH) expression was investigated, and then different DO control strategies were tested for effects on GDH expression. DO control strategy IV (50% 0–9 h, 30% 9–18 h) provided the highest cell density (15.43 g/L) and GDH concentration (3.42 g/L), values 1.59- and 1.99-times higher than those achieved at 10% DO. The accumulation of acetate was 2.24 g/L with DO control strategy IV, a decrease of 40.74% relative to that achieved for growth at 10% DO. Additionally, under DO control strategy IV, there was lower expression of PoxB, a key enzyme for acetate synthesis, at both the transcriptional and translational level. At the same time, higher transcription and protein expression levels were observed for a glyoxylate shunt gene (*aceA*), an acetate uptake gene (*acs*), gluconeogenesis and anaplerotic pathways genes (*pckA*, *ppsA*, *ppc*, and *sfcA*), and a TCA cycle gene (*gltA*). The flux of acetate with DO strategy IV was 8.4%, a decrease of 62.33% compared with the flux at 10% DO. This decrease represents both lower flux for acetate synthesis and increased flux of reused acetate.

Keywords *Streptococcus suis* · Glutamate dehydrogenase · *Escherichia coli* · Acetate · Dissolved oxygen

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Introduction

Streptococcus suis (*S. suis*) is a Gram-positive, facultative anaerobic bacterium that is economically important, because it causes a wide range of diseases in pigs (Lun et al. 2007). The glutamate dehydrogenase (GDH) of *S. suis* is an effective antigen targeted by a vaccine that protects swine from *S. suis* infection, and GDH is also used as the target gene in serological assays to diagnose *S. suis* infection (Kutz and Okwumabua 2008; Wang et al. 2020). In our previous study, we constructed a recombinant strain of *E. coli*, SSGPA by deletion of *pta-ackA* from *E. coli* strain SSG. SSGPA expresses a high level of GDH and a decreased concentration of acetate, but previous work had shown that the accumulation of acetate in *E. coli* inhibits cell growth and GDH expression (Wang et al. 2020; Parimi et al. 2017; Dong et al. 2017).

Escherichia coli generates acetate as an undesirable byproduct with negative effects on cell growth and

expression of recombinant protein (Bemal et al. 2016; Rajaraman et al. 2016), and an effective strategy to optimize protein production is to limit acetate accumulation (Eiteman and Altman 2006). Dissolved oxygen (DO) is a key parameter in fermentation, affecting cytochrome activity and enzymes related to the glycolytic (EMP) pathway, the pentose phosphate (PP) pathway, and the tricarboxylic acid (TCA) cycle (Zheng and Kwon 2013). Higher bacterial cell concentration is achieved by controlling the level of DO (Negrete and Shiloach 2017). The concentration of acetate is accumulated mainly through the phosphotransacetylase (*pta*)-acetate kinase (*ackA*) pathway and the pyruvate oxidase B (*poxB*) pathway; both pathways are also affected by gluconeogenesis (Kumar and Shimizu 2010). Acetate consumption occurs by its conversion back to acetyl-CoA by acetyl-CoA synthetase (*acs*), which is the reverse action of Pta-AckA and the glyoxylate shunt pathway (*aceBAK*) (Rajaraman et al. 2016; Wang et al. 2013). Thus, the concentration of accumulated acetate during cultivation of *E. coli* is the combined result of acetate formation and consumption (Phue and Shiloach 2005).

A well-known disadvantage of oxygen limitation during cultivation is an increased rate of acetate accumulation relative to that under aerobic conditions (Veeravalli et al. 2018). Acetate accumulation is inversely correlated to DO levels, with enhanced acetate production at lower DO levels due to decreased TCA cycle activity and changes in the transcriptional levels of genes associated with glucose and acetate metabolism (Phue and Shiloach 2005; Zhao et al. 2020). Northern blot analysis of proteins that act in the acetate synthesis pathway indicated that only the Pta-AckA pathway genes are transcribed at high oxygen levels, but at low oxygen levels, these Pta-AckA pathway genes and PoxB are transcribed (Phue and Shiloach 2005). Analysis of L-tryptophan production by *E. coli* at different DO levels revealed high transcription levels of key genes for tryptophan biosynthesis and low transcription levels of key genes involved in acetate synthesis at a relative high DO level, leading to reduced acetate excretion and improved L-tryptophan production (Zhao et al. 2020). However, the concentration of reactive oxygen species (ROS) increases with increasing DO concentration during *E. coli* growth, and high levels of ROS results in irreversible damage to cellular components (Baez and Shiloach 2013).

In this study, the effect of different DO levels on GDH expression by *E. coli* SSGPA was investigated, and then different DO control strategies were tested for effects on the expression of GDH. The transcription levels and enzyme activities of key genes for acetate synthesis were analyzed, and the metabolic flux distribution of key nodes included in the acetate synthesis pathway were determined to explain the low excretion of acetate and high expression of GDH at an optimized DO control level.

Materials and methods

Strain and media

The strain *E. coli* SSGPA used for GDH expression in this study was constructed from *E. coli* SSG by deletion of *pta-ackA* as described in our previous work (Wang et al. 2020). The strain was maintained on Luria–Bertani (LB) agar containing 100 mg/L of ampicillin (Am).

The seed medium for GDH expression was Luria Broth (LB), with the following components (in g/L): tryptone 10, yeast extract 5, and NaCl 10. The fermentation medium for GDH expression had the following composition (in g/L): glucose 10, tryptone 5, yeast extract 10, $(\text{NH}_4)_2\text{SO}_4$ 2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 2.5, KH_2PO_4 1.5, citrate 2, FeSO_4 0.1, and betaine 1.5. The pH value of the seed and fermentation media was adjusted to 7.0 by addition of 4 mol/L NaOH solution.

Culture condition

The expression of GDH protein by *E. coli* SSGPA was carried out in a 10-L fermenter (Biotech 2012, Shanghai Bailun Biotech Co., Ltd., Shanghai, China). A sample of 600 mL seed medium in a 3000 mL baffled flask was inoculated with a single colony of *E. coli* SSGPA and cultivated at 35 °C by shaking at 200 rev/min for 12 h. This culture grown in the baffled flask was then inoculated aseptically (10% v/v) into 6 L of fermentation medium in a 10-L fermenter. The temperature and pH were controlled at 35 °C and 7.0, respectively, while DO levels were maintained to meet experimental requirements by adjusting the agitation and aeration rates. Once the cell density (OD_{600}) increased to about 10.0, 0.5 mmol/L isopropyl thiogalactose (IPTG) was added into the fermenter to induce GDH expression. When the initial glucose was depleted, glucose solution (50%, w/v) was added to the fermenter according to the DO feedback strategy (Zhao et al. 2016).

DO control strategy

The DO levels during GDH expression by *E. coli* SSGPA were controlled at 10%, 20%, 30%, 50%, or 100%. Four DO control strategy were developed and tested for the expression of GDH protein: strategy I [20% (0–9 h), 30% (9–18 h)], strategy II [30% (0–9 h), 20% (9–18 h)], strategy III [50% (0–9 h), 20% (9–18 h)], and strategy IV [50% (0–9 h), 30% (9–18 h)].

Transcriptome analysis of *E. coli*

The total RNA of *E. coli* samples during mid-logarithmic growth of GDH expression was extracted using the RNAiso Plus kit (Takara, China), and transcriptome analysis of *E. coli* with different DO levels was performed as described

previously (Zhao et al. 2020; Mortazavi et al. 2008). The transcription of several genes involved in glucose and acetate metabolism were examined, including an acetate formation gene (*poxB*), a glyoxylate shunt gene (*aceA*), an acetate uptake gene (*acs*), gluconeogenesis and anaplerotic pathways genes (*pckA*, *ppsA*, *ppc*, and *sfcA*), and a TCA cycle gene (*gltA*) (Phue and Shiloach 2005).

Activity analysis of key enzymes for acetate synthesis

Crude extracts were prepared from cells harvested during the mid-logphase (10 h) of fermentation (Tan et al. 2016). *E. coli* cells were collected by centrifugation at 8000×g for 10 min at 4 °C, washed twice with 50 mM Tris buffer (pH 7.0), and then suspended in the same buffer with 1× protease inhibitor (Roche, Switzerland) to an OD₅₅₀ of 10 for sonication treatment. After centrifugation at 12,000×g and 4 °C for 20 min, the supernatant (crude extract) was transferred to a new tube (Piao et al. 2019). The activities of key enzymes for acetate synthesis were then measured as described previously, including fructose biphosphatase (Fbn), phosphoenolpyruvate carboxykinase (Pck), phosphoenolpyruvate synthase (Pps), Phosphoenolpyruvate carboxylase (Ppc), citrate synthase (Glt), pyruvate oxidase B (PoxB), and acetyl-CoA synthase (Acs) (Lin et al. 2018; Zhang et al. 2018; Zhao et al. 2018).

Metabolic flux distribution analysis of key nodes for acetate synthesis

Based on the law of conservation and the pseudo-steady-state hypothesis (PSSH) of the intracellular intermediate metabolites, a mass metabolic matrix was constructed (Chen and Zeng 2017). The metabolic network and the reaction rate equations of metabolic nodes were established as previously described (Bemal et al. 2016; Castaño-Cerezo et al. 2009). According to the analysis of metabolic flux balance and the stoichiometry model, and based on the strain being in stationary phase near the end of the fermentation period, the metabolic flux was determined for key nodes in the acetate synthesis pathway with different DO levels during the later period of GDH expression using MATLAB (MathWorks, Natick, MA, USA) (Enjalbert et al. 2017; Wang et al. 2019).

Analysis of fermentation products

The cell density of *E. coli* SSGPA during the GDH expression process was measured by spectrophotometry (OD₆₀₀), and the dry cell weight (DCW) was assessed using the following relationship: 1 OD₆₀₀ = 0.42 g/L DCW (Zhao et al. 2020). The concentration of glucose was determined using an SBA-40E biosensor analyzer (Biology Institute of

Shandong Academy of Sciences, Jinan, China). The acetate concentration was determined by high-performance liquid chromatography using an Agilent 1200 system (Agilent Technologies, Santa Clara, CA, USA) equipped with an Aminex HPX-87H (Bio-Rad Laboratories, Inc., USA) (Lin et al. 2016). The concentration of GDH protein was determined as described previously (Wang et al. 2020).

Statistical analysis

All experiments were conducted in triplicate, and the data were averaged and are presented as mean values ± standard deviation. One-way analysis of variance was followed by Tukey test for multiple comparison to determine significant differences. Statistical significance was defined as $p < 0.05$.

Results

GDH expression with different DO levels

Cell density and GDH expression levels

The cell density and GDH expression levels were determined for *E. coli* SSGPA cultivated at different DO levels (10%, 20%, 30%, 50%, and 100%), as presented in Fig. 1. For DO levels maintained below 50%, the cell density and GDH concentration increased with higher DO level. The highest cell density (13.63 g/L) and GDH concentration (3.05 g/L) were obtained at a DO level of 50%, increased values by 40.08% and 40.55%, respectively, compared with those at 10% DO (9.73 g/L and 2.17 g/L). The cell density values at 20% and 30% DO were 12.25 g/L and 13.29 g/L, respectively, with GDH concentrations of 2.78 g/L and 2.98 g/L, respectively. The cell density and GDH concentration at 50% DO were significantly different from those at 10% and 20% ($p < 0.05$), and those at 30% and 50% were not significantly different. For cultivation at 100% DO, cell density and GDH concentration were lowest, with values of 9.41 g/L and 2.38 g/L, respectively.

Accumulation and synthesis rate of acetate

Figure 2 shows the accumulation of acetate by *E. coli* SSGPA and its synthesis rate at different DO levels. For GDH expression, acetate accumulated during the early fermentation period (0–12 h), and then the accumulated acetate was partially consumed during the later fermentation phase (12–18 h). The accumulation of acetate and acetate synthesis rate both decreased during the first ten hours of culture time with increasing DO levels. The accumulation of acetate at the end of fermentation with 10% DO (3.78 g/L) was highest, while that with 100% DO

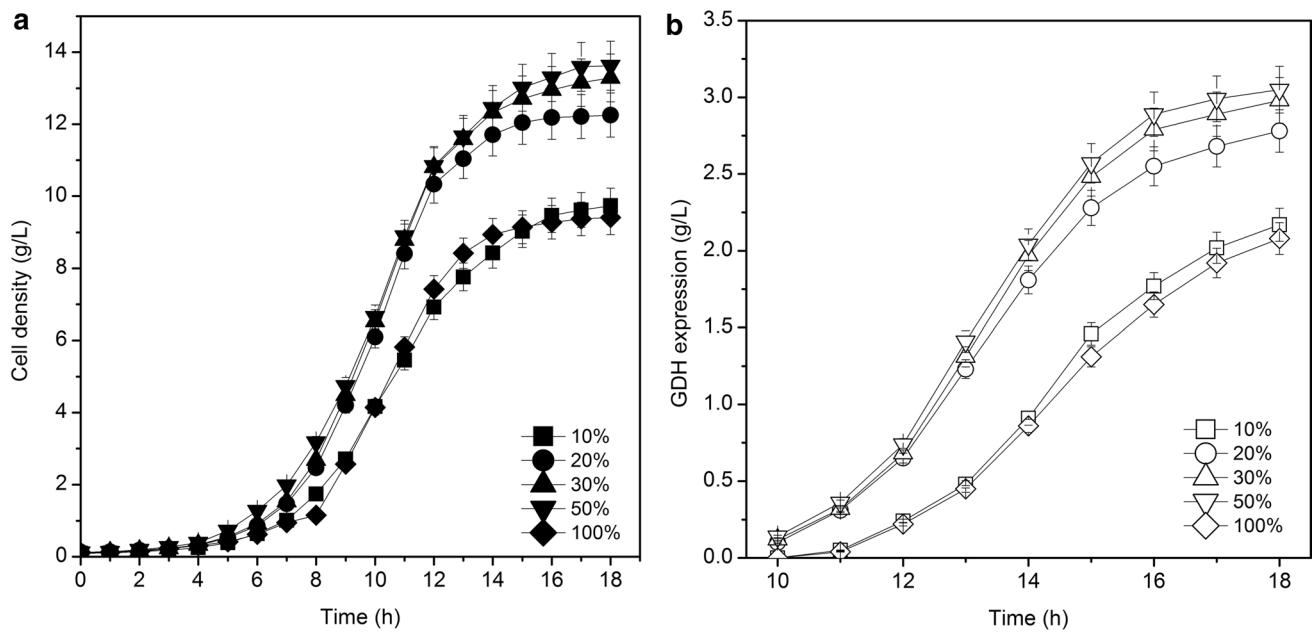


Fig. 1 *Escherichia coli* SSGPA cell density and GDH expression at different DO levels ($p < 0.05$)

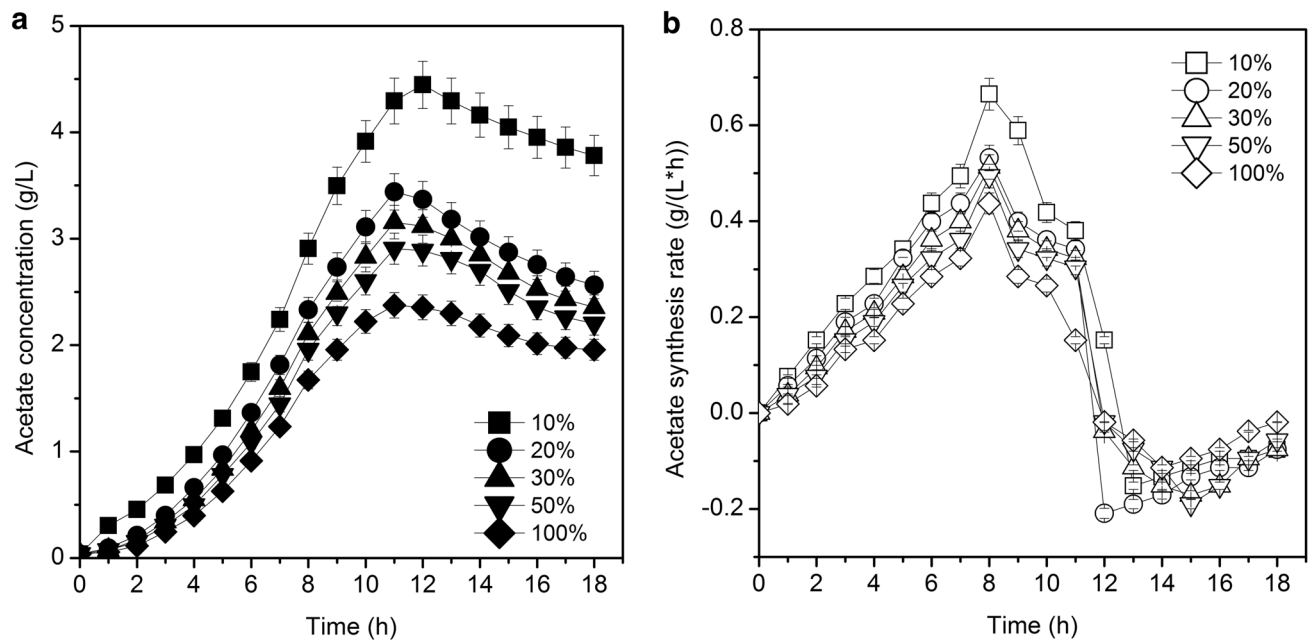


Fig. 2 *Escherichia coli* SSGPA acetate accumulation and acetate synthesis rate at different DO levels. Reported acetate synthesis rates below zero are due to acetate consumption ($p < 0.05$)

(1.96 g/L) was lowest. The concentration of acetate accumulated at 50% DO (2.20 g/L) at the end of cultivation was decreased by 14.06% and 6.78% compared with that at 20% and 30% DO. The accumulation of acetate at 100% DO level was significantly different from that at other DO levels ($p < 0.05$).

GDH expression with different DO control strategies

Cell density and GDH expression level

Based on the above results, the four DO control strategies were applied for GDH expression by *E. coli* SSGPA, and

the resulting cell density and GDH expression levels are displayed in Fig. 3. The cell density (15.43 g/L) and GDH concentration (3.42 g/L) obtained with DO control strategy IV were highest, with increases of 5.98% and 7.89%, respectively, compared with those using DO control strategy III (14.56 g/L and 3.17 g/L). The cell density and GDH concentration with DO control strategy I (12.55 g/L and 2.83 g/L, respectively) was not significantly different from those obtained with DO control strategy II (12.92 g/L and 2.89 g/L, respectively). However, the cell density and GDH concentration with DO control strategy III and IV were significantly different from those obtained with DO control strategies I and II ($p < 0.05$).

Accumulation and production rate of acetate

The accumulation of acetate for different DO control strategies during GDH expression by *E. coli* SSGPA is shown in Fig. 4, along with the acetate synthesis rate. Lower concentrations of acetate accumulated at high DO levels, and the acetate synthesis rate increased with lower DO levels during early fermentation. The average values for the accumulation of acetate with DO control strategies I, II, III, and IV at the end of fermentation were 2.49 g/L, 2.41 g/L, 2.30 g/L, and 2.24 g/L, respectively. The accumulated acetate concentrations at the end of fermentation with DO control strategies III and IV were significantly different from those obtained using DO control strategies I and II ($p < 0.05$).

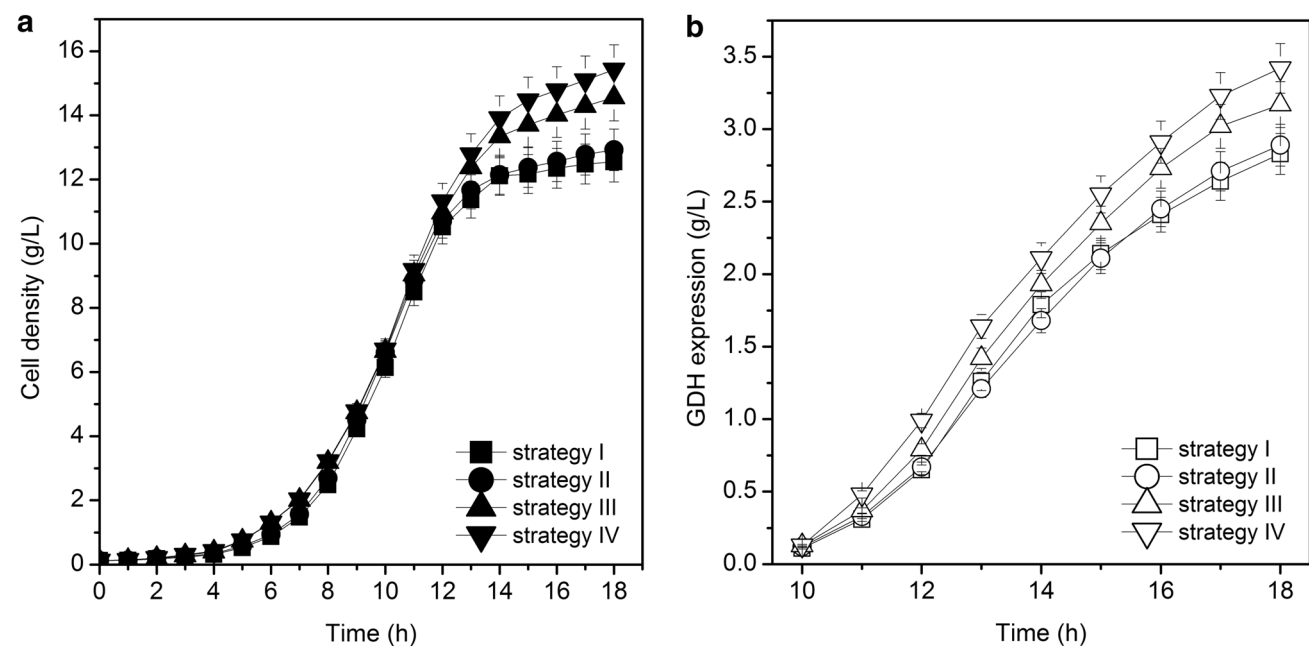


Fig. 3 *Escherichia coli* SSGPA cell density and GDH expression levels for different DO control strategies ($p < 0.05$)

Mechanism analysis of acetate synthesis at different DO levels

Transcription levels of key genes for acetate synthesis

Based on the above results, due to the oxygen limitation with 10% DO level and the more appropriate DO level with DO control strategy IV, higher and lower concentration of acetate were accumulated, leading to lower and higher expression of GDH, respectively. Therefore, the acetate synthesis mechanism with 10% DO level and DO strategy IV were selected to investigate the effect of DO level on acetate synthesis. The transcription levels of key genes for acetate synthesis were measured at 10 h cultivation time for cultivation using DO strategy IV or a 10% DO level, as shown in Table 1. There were significant changes in the transcription levels of key genes for acetate synthesis at different DO levels. When the DO level was maintained at 30% using DO control strategy IV at 10 h, the transcription level of an acetate-producing gene (*poxB*) was lower, while levels of acetate-utilizing genes (*acs* and *aceA*) were higher. Additionally, higher transcription levels were observed for a TCA cycle-associated gene (*gltA*) and genes associated with gluconeogenesis and the anaplerotic pathway (*pckA*, *ppsA*, *ppc*, and *sfcA*).

Activity of key enzymes for acetate synthesis

Figure 5 shows the activities of key enzymes for acetate synthesis in cultures grown for GDH expression with DO control

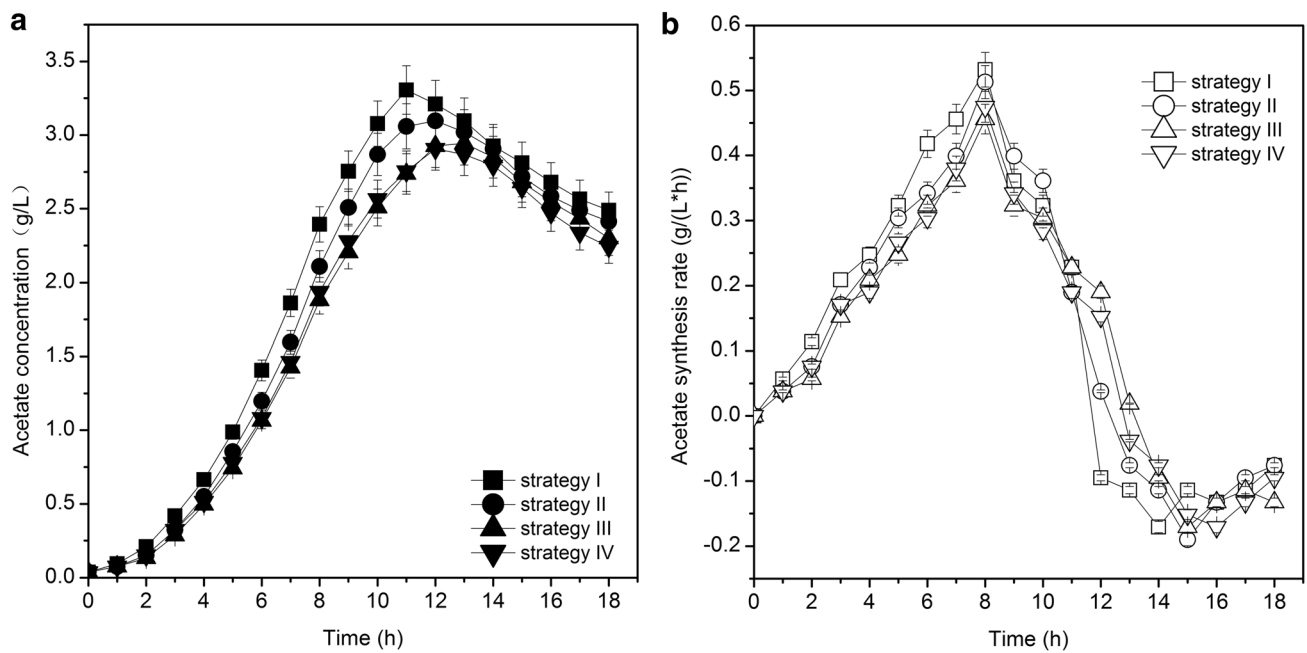


Fig. 4 *Escherichia coli* SSGPA acetate accumulation and acetate synthesis rate for different DO control strategies. Reported acetate synthesis rates below zero are due to acetate consumption ($p < 0.05$)

Table 1 Transcription levels of key genes for acetate synthesis at 10 h culture time using DO control strategy IV compared to level at 10% DO

Gene	Transcription ratio	Enzyme
<i>fbn</i>	0.96-times (↓)	Fructose-bisphosphatase
<i>poxB</i>	1.32-times (↓)	Pyruvate oxidase B
<i>aceA</i>	1.24-times (↑)	Isocitrate lyase
<i>acs</i>	1.19-times (↑)	Acetyl-CoA synthetase
<i>pckA</i>	1.21-times (↑)	Phosphoenolpyruvate carboxykinase
<i>ppsA</i>	1.12-times (↑)	Phosphoenolpyruvate synthase
<i>ppc</i>	1.18-times (↑)	Phosphoenolpyruvate carboxylase
<i>sfcA</i>	1.21-times (↑)	NAD-linked Malate dehydrogenase
<i>gltA</i>	1.09-times (↑)	Citrate synthase

strategy IV or 10% DO. The activity of a key enzyme of the EMP pathway, Fbn, was decreased 6.48% with DO strategy IV compared to its activity at 10% DO. The activities of PoxB were 154 U/g and 196 U/g with DO strategy IV and 10% DO, respectively. Using DO strategy IV, the activities of Acs, Pck, Pps, Ppc, and Glt were increased by 13.71%, 14.97%, 18.60%, and 10.71%, respectively, compared to activities at 10% DO.

Metabolic flux distribution of key nodes for acetate synthesis

The metabolic flux distribution of key nodes for acetate synthesis was determined using DO control strategy IV and 10%

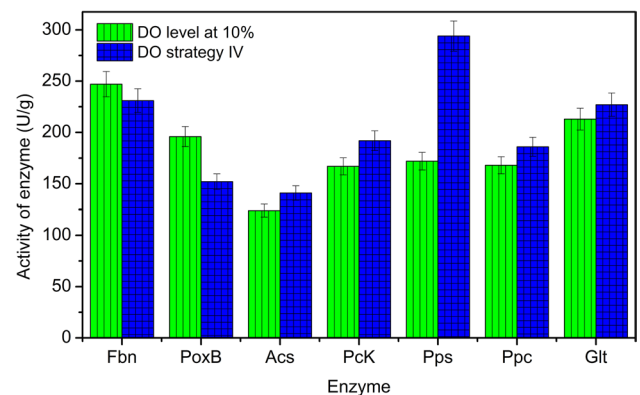
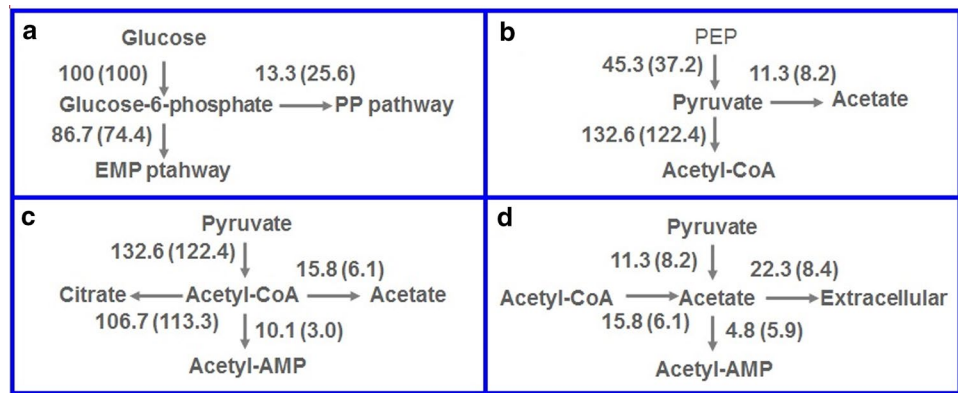


Fig. 5 The activities of key enzymes for acetate synthesis at 10 h of GDH expression with DO control strategy IV and 10% DO ($p < 0.05$)

DO during the later fermentation period (12–18 h) of GDH expression by *E. coli* SSGPA, and the results are presented in Fig. 6. The DO level affected the distribution of carbon metabolic flux. The carbon flux entering the EMP pathway was 86.7% at 10% DO and decreased to 74.4% for DO strategy IV. The flux for pyruvate synthesis from PEP with DO strategy IV was decreased by 17.88% compared with that at 10% DO, and the flux of pyruvate for acetyl-CoA and acetate formation with DO strategy IV also decreased by 7.69% and 27.43%, respectively, compared to those at 10% DO. Using DO strategy IV, more flux of acetyl-CoA went towards citrate formation, with lower flux of acetyl-CoA to formation of acetyl-AMP and acetate. With DO strategy

Fig. 6 The metabolic flux distribution of key nodes for acetate synthesis with DO control strategy IV and 10% DO during the later fermentation period (12–18 h) of GDH expression. The values in bracket indicate metabolic flux with DO control strategy IV



IV, there was 5.9% carbon flux from acetate to acetyl-AMP synthesis, an increase of 12.29% compared with that at 10% DO. With lower flux for acetate synthesis and greater flux of acetate reused, the overall flux of acetate with DO strategy IV reduced to 8.4%, a decrease of 62.33% compared with that at 10% DO.

Discussion

Acetate overflow can reduce cell density and product yields (Bemal et al. 2016), and the variation of DO level could potentially alter bacterial metabolism and acetate formation, affecting both cell growth and recombinant proteins production capability (Phue and Shiloach 2005). Performing GDH expression at different DO levels, the excretion of acetate decreased with higher DO levels, indicating that the accumulation of acetate was inversely correlated to DO level (Zhao et al. 2020). The inhibition of cell growth and protein formation caused by acetate decreased due to reduced acetate excretion, and cell density and GDH concentration increased with DO level below 50%. However, the lowest cell density and GDH expression level were obtained when the lowest concentration of acetate was accumulated at a DO level of 100%, because the formation of ROS with high oxygen concentration caused cellular damage by altering enzymes, proteins, and DNA (Negrete and Shiloach 2017; Rui et al. 2010). For example, the expression level of porcine circovirus type 2 capsid protein by *E. coli* was lowest when the DO level was maintained at 100% (Cheng et al. 2015). Due to the oxygen limitation with DO level of 10%, the highest concentration of acetate was accumulated, leading to lower cell density and GDH expression. High cell density and GDH expression level were obtained with relatively high DO levels, and both cell density and GDH expression were higher with DO control strategies III and IV than those at 50% DO, which revealed that a DO level of 20% or 30% was better for GDH formation than 50%. Overall, the highest cell density and GDH concentration were obtained with

DO control strategy IV because of the appropriate DO level, with values 1.59- and 1.99-times higher than those obtained when cultured at 10% DO, which showed that DO level was the key factor for GDH expression. In addition, cell density and GDH expression with DO control strategy IV were increased by 11.32% and 11.21% compared with those of 50% DO level, which were significantly different ($p < 0.05$).

Compared to the early fermentation period, there was decreased acetate accumulation with GDH expression during the later culture phase. Acetate can be consumed as a carbon and energy source to support cell growth after consumption of glucose, and the concentration of glucose reached zero when a DO feedback feeding strategy was used (Zhao et al. 2016). A previous study found 10 g/L of acetate accumulated in a strain deleted for *ackA*, but this amount decreased to 5.0 g/L at the end of fermentation, which was consistent with the change of acetate concentration during GDH expression (Parimi et al. 2017). Acs is a high-affinity enzyme that scavenges low acetate concentrations upon depletion of the main carbon source (Bemal et al. 2016; Pinalhal et al. 2019), and the overexpression of Acs has been proposed as a potential strategy to reduce acetate accumulation (Parimi et al. 2017). During continuous culture, the expression of *acs* can lead to a futile cycle of simultaneous acetate production and scavenging, thus limiting acetate overflow (Bemal et al. 2016). The higher transcription level of *acs* and activity of Acs with DO control strategy IV increased the flux from acetate to acetyl-AMP synthesis, with decreased concentration of acetate compared to that at 10% DO level.

The PoxB pathway is the major pathway for acetate formation during the stationary phase, and deletion of *poxB* decreased the accumulation of acetate (Bemal et al. 2016). The lower transcription level of *poxB* and activity of PoxB resulted in a lower excretion of acetate (Phue and Shiloach 2005). The increasing transcription levels of *ppsA*, *pckA*, *ppc*, and *sfcA* and increasing enzyme activities of Pck, Pps, and Ppc could decrease the concentrations of pyruvate and Acetyl-CoA, and the synthesis flux of pyruvate and Acetyl-CoA at higher DO level (Phue and Shiloach 2005; Yang

et al. 2018). Acetyl-CoA is a key substrate for acetate synthesis, with most acetyl-CoA entering the TCA cycle, leading to its complete oxidation into CO₂ (Bemal et al. 2016). The unbalance between carbon source catabolism and cellular respiration forms a bottleneck at the pyruvate node and the accumulation of acetyl-CoA, leading to improved acetate accumulation (De Mets et al. 2019). As a result, higher transcription levels of *aceA* and *gltA* and increased enzyme activity of Glt increased the TCA cycle activity with DO control strategy IV, resulting in the accumulation of less acetate compared with that of 10% DO level.

Acetate is considered a typical “overflow” metabolite when the carbon flux into central metabolic pathways exceeds the cellular biosynthetic demands and capacity for energy generation (Parimi et al. 2017). The excretion of acetate can be affected by the flux ratio between the EMP and PP pathways, and acetate yield increased with increased EMP flux (Kamata et al. 2019). Due to the decreased flux for the EMP pathway caused by the transcription level of *fbn* and activity of Fbn with DO control strategy IV, there was less accumulation of acetate compared with cultivation at DO level of 10%. Additionally, the increased transcription of genes related to gluconeogenesis and anaplerotic pathways and increased TCA cycle capacity at higher DO level resulted in less flux for pyruvate and acetyl-CoA synthesis and greater consumption of acetyl-CoA by the TCA cycle (Song et al. 2018). With lower flux for acetate synthesis and increased flux of acetate reused with DO control strategy IV, the overall flux of acetate was lower, and the accumulation of acetate was decreased by 40.74% compared with that at 10% DO level. According to the analysis of acetate synthesis with different DO levels, the accumulation of acetate was decreased obviously with an appropriate DO level, increasing both cell density and GDH expression.

Conclusion

DO level was identified as a key parameter for acetate synthesis and GDH expression. The transcriptional level of key genes, activity of a key enzyme, and metabolic flux of key nodes for acetate synthesis were all affected by DO level. Using DO control strategy IV (50% 0–9 h, 30% 9–18 h), the transcriptional level of an acetate-producing gene was lower, while levels of acetate-utilizing genes, a TCA cycle-associated gene, genes associated with gluconeogenesis and the anaplerotic pathway were higher, compared with the levels obtained at DO level of 10%. Additionally, the activities of key enzymes for acetate synthesis with DO control strategy IV or 10% DO level were consistent with the transcription level variation of these genes. The flux of acetate synthesis with DO strategy IV was 8.4%, a 62.33% decrease compared with that obtained using 10% DO level. Using the

appropriate DO level with DO control strategy IV, the accumulation of acetate decreased to 2.24 g/L, which resulted in 15.43 g/L cell density and 3.42 g/L GDH concentration, amounts that were 1.59- and 1.99-times higher than those achieved at 10% DO level.

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Author contributions LC, XX and ZS designed the research. LC and XX performed the experiments. LC, CZ, ZS, and CL acquired and analyzed the data. XZ, JW, JW, and LW contributed to writing of the manuscript. Finally, all authors have reviewed and approved the final submitted manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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