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ORIGINAL ARTICLE

Improvement of the production of L-tryptophan in *Escherichia coli* by application of a dissolved oxygen stage control strategy

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Abstract Dissolved oxygen (DO) is a key parameter for the production of L-tryptophan, and maintenance of appropriate DO levels can be used to increase the formation of L-tryptophan and reduce the accumulation of acetate and glutamate. In addition, controlling the level of DO by adjusting the feeding rate of glucose solution affects the concentration of glucose in the fermentation process. In this study, the effects of DO levels on L-tryptophan production were investigated, and four strategies of DO stage control were developed for use in L-tryptophan fermentation. The results indicate that the application of DO stage control I (20 % oxygen at 0–20 h, 30 % at 20–38 h) during L-tryptophan fermentation resulted in the highest dry cell weight (51.2 g/L) and production of L-tryptophan (46.8 g/L), which were 1.10 and 1.28 times as high, respectively, as those obtained during DO stage control III (50 % oxygen at 0–20 h and 20 % at 20–38 h). Additionally, the total glucose conversion percentage with DO stage control I was 17.2 %, which was 13.16 % higher than that of DO stage control III. Furthermore, the metabolic flux distribution with DO stage control I and III revealed greater carbon flux into the pentose phosphate pathway, while the flux of byproducts (acetate, glutamate, and lactate) was lower during DO stage control I. Finally, the flux of tryptophan with DO stage control I was 19.3 %, which was 1.79 times as high as that obtained with DO stage control III.

Keywords L-Tryptophan · *Escherichia coli* · Dissolved oxygen · Acetate · Glutamate · Metabolic flux

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Introduction

L-Tryptophan is an essential aromatic-group amino acid for humans and other animals, and is widely used in the food, animal feed, and pharmaceutical industries (Zhao et al. 2011; Wang et al. 2013a). The most common method for producing L-tryptophan is through microbial fermentation using *Corynebacterium glutamicum* and *Escherichia coli* from inexpensive and renewable carbohydrates such as sucrose or glucose (Ikeda 2006; Liu et al. 2012). *C. glutamicum* is a well-established microorganism for biotechnological applications, and has been engineered for the production of important amino acids such as L-glutamate and L-lysine (Kulis-Horn et al. 2014). In light of the relatively small genome/proteome size and low manufacturing costs of *E. coli*, it has become the organism of choice for the production of a wide variety of products for therapeutic, diagnostic, and industrial applications (Eiteman and Altman 2006; Mostovenko et al. 2011). Studies have shown that the use of *E. coli* in L-tryptophan production results in higher yields and lower production costs than those achieved with *C. glutamicum*, and thus *E. coli* is the preferred strain for the production of L-tryptophan (Ikeda 2006; Wang et al. 2013a). Fermentative

production of L-tryptophan by *E. coli* has been investigated extensively, and production is clearly improved by increasing the supply of precursor for tryptophan biosynthesis and reducing the accumulation of acetate (Dodge and Gerstner 2002; Shen et al. 2012; Wang et al. 2013b).

Acetate is a primary inhibitory metabolite in cultures of *E. coli*, and acetate metabolism plays an important role in controlling central metabolism and bioprocess performance (Castaño-Cerezo et al. 2009). The accumulation of acetate can be reduced by optimization of culture conditions and genetic modification, and most measures to overcome acetate formation ultimately bring the growth rate and oxygen consumption into better balance (Eiteman and Altman 2006). Two pathways contribute to acetate formation: the phosphotransacetylase-acetate kinase (Pta -AckA) pathway and the pyruvate oxidase B (PoxB) pathway (Cheng et al. 2012). The mutants with deletion of the genes encoding Pta, AckA or PoxB accumulate lower concentrations of acetate than the parental strain (De Mey et al. 2007; Zhao et al. 2015). Northern blot analysis has shown that only the Pta-AckA pathway genes are transcribed with high oxygen levels, while genes of both the Pta-AckA and PoxB pathways appear to be transcribed at low oxygen levels (Phue and Shiloach 2005). In addition, research has shown that enhanced acetate accumulation at lower DO levels is the result of lower tricarboxylic acid (TCA) cycle activity and altered transcription levels of genes associated with glucose and acetate metabolism, and that the accumulation of acetate is reduced by maintaining high DO levels (Phue and Shiloach 2005). However, increased DO concentrations during *E. coli* growth stimulate an increase in the intracellular concentration of reactive oxygen species (ROS), and high levels of ROS are known to cause stress to *E. coli*, resulting in irreversible damage to cellular components (Baez and Shiloach 2013). Under the superoxide stress conditions caused by paraquat in *E. coli* cultures, the fluxes in the glyoxylate shunt were found to increase significantly, while the fluxes associated with the TCA cycle decreased, and global flux changes resulted in the accumulation of α-ketoglutarate (Rui et al. 2010). Glutamate production is increased by improving the accumulation of α-ketoglutarate, which has a negative impact on the production of L-tryptophan (Dodge and Gerstner 2002). The transcription factors OxyR and SoxRS have been identified as key regulators protecting against the effects of ROS, and the activation of these regulators greatly increases cellular resistance to oxidizing agents (Pomposiello and Demple 2001). Thus, maintaining appropriate DO levels is important for reducing the excretion of acetate and avoiding the inhibition caused by high ROS concentrations.

A number of enzymes of the Embden–Meyerhof–Parnas (EMP), pentose phosphate (PP), and TCA cycle pathways, together with cytochrome measurements, have been reported to be affected by DO level (Thomas et al. 1972). Metabolic flux analysis of L-threonine biosynthesis with different levels of DO showed that with a DO level of 20 %, the flux of carbon entering the PP pathway was 58.08 %, which was 2.28 times as high as that observed at a 5 % DO level (Huang et al. 2008). The production of L-tryptophan increased as the metabolic flux of the EMP pathway was redirected to the PP pathway, which improved the supply of erythrose-4-phosphate (E4P) (Huang et al. 2011). The increase in carbon flux through the oxidative branch of the PP pathway and the concomitant increase in nicotinamide adenine dinucleotide phosphate (NADPH) production were found to have a positive impact, improving the biosynthesis of aromatic compounds (Báez-Viveros et al. 2007). The transcription of phosphoenolpyruvate carboxykinase (*pckA*) and phosphoenolpyruvate synthase (*ppsA*) related to gluconeogenesis occurred at higher rates with high DO levels than low DO levels (Phue and Shiloach 2005). The overexpression of gluconeogenic genes significantly improved the yield of aromatic amino acids, presumably by increasing the availability of phosphoenolpyruvate (PEP) (Báez-Viveros et al. 2007), and the production of L-tryptophan was increased by improving the availability of PEP and E4P (Shen et al. 2012). A decrease in the glucose consumption rate was found to occur at high DO levels (Baez and Shiloach 2013), and reduction in glucose uptake greatly improved PEP availability due to the PEP consumed by the transport of glucose in *E. coli* cultures (Gosset 2005). Enhancement of the supply of PEP and E4P by an appropriate DO level has a positive impact on the formation of L-tryptophan.

Many fed-batch strategies have been developed with the express purpose of preventing acetate accumulation, and the most effective means of reducing acetate excretion is through the maintenance of glucose concentration and specific growth rate below the threshold value by adjusting the feeding rate of limiting nutrients (Cheng et al. 2012). DO-stat feeding can be used to avoid substrate overfeeding and O_2 limitation. The use of a DO sensor to detect and avoid overflow metabolism, combined with a safety net to guarantee aerobic conditions, can help to avoid the accumulation of acetate in *E. coli* cultures (Åkesson et al. 2001). The use of a balanced DO-stat strategy with a computer control system during the production of phenylalanine was successful in keeping the excretion of acetate at zero throughout the cultivation period, and both cell density and production of phenylalanine increased markedly (Konstantinov et al. 1990). The DO level can be maintained at a certain value by adjusting the glucose feeding rate through the application of a DO feedback algorithm (Cheng et al. 2012). In the production of L-tryptophan, optimization of the glucose feed rate can increase production rates and limit the formation of byproducts (Dodge and Gerstner 2002). Different glucose feeding rates result in glucose concentrations that vary with the level of DO. The glucose

concentration is an important factor for the formation of tryptophan, and higher L-tryptophan yields can be obtained with an appropriate glucose concentration (Cheng et al. 2012). In the present study, the DO feedback feeding strategy was used in the production of L-tryptophan. Taking into account the impact of DO level on the formation of byproducts and the availability of PEP and E4P, the effects of DO levels on L-tryptophan production were investigated, and the results were used to develop DO stage control strategies for application to L-tryptophan fermentation. In addition, the metabolic flux distribution of tryptophan biosynthesis with different DO stage control strategies was analyzed.

Materials and methods

Microorganism and medium

The L-tryptophan-producing *E. coli* TRTH (*trpEDCBA* + Tet^R, Δ*tnaA*) strain used in this study was obtained from an earlier study conducted by our group (Liu et al. 2012), which was stored in the culture collection of Tianjin University of Science and Technology (Collection number: TCCC 27006). During storage, the organism was maintained on Luria–Bertani agar containing 50 mg/L tetracycline (Tet).

The seed medium contained the following components (in g/L): glucose (20), yeast extract (10), $(NH_4)_2SO_4$ (8), sodium citrate (1.5), $MgSO_4{\cdot}7H_2O$ (2.5), KH_2PO_4 (2.5), $FeSO_4{\cdot}$ 7H2O (0.15), vitamin B_1 (0.05), and Tet (0.05). The fermentation medium used to produce L-tryptophan contained the following components (in g/L): glucose (10), yeast extract (1), $(NH_4)_2SO_4$ (4), sodium citrate (1), $MgSO_4{\cdot}7H_2O$ (2.5), KH_2PO_4 (2), and $FeSO_4{\cdot}7H_2O$ (0.1). The pH of both seed and production media was adjusted to 7.0 using 4 mol/L NaOH.

Culture conditions

Fermentation was performed in 50-L fermenters (BIOTECH-2002 bioprocess controller; Baoxing, Shanghai, China). A 500-mL baffled flask (Shuniu, Chengdu, China) containing 30 mL of seed medium was inoculated with a single colony of *E. coli* TRTH, then cultivated at 35 °C with shaking at 200 rpm for 12 h. A 30-mL inoculum of this culture was added aseptically to a 5-L seed fermenter (BIOTECH-2002 bioprocess controller; Baoxing, Shanghai, China) containing 3 L of seed medium, which was then cultivated at 35 °C for 16 h. The culture grown in the seed fermenter was inoculated aseptically (10 % v/v) into 25 L of production medium in a 50-L fermenter, after which the pH was adjusted to 7.0 with 25 % ammonium hydroxide (w/w). The temperature was maintained at 35 °C during the cultivation period, while the DO was adjusted to different levels to meet specific experimental requirements. When the initial glucose was depleted, additional glucose (80 % w/v) was fed into the fermenter according to the DO feedback feeding strategy.

DO control strategy

The concentration of DO in the fermentation process was measured automatically with DO electrodes attached to the fermenters, and DO levels were controlled at different values by adjusting the agitation and aeration rates. If the maximum agitation and aeration rates could not maintain the desired DO, the levels were controlled by adjusting the feeding rate of the glucose solution. First, the DO levels were maintained at 5, 20, 30, and 50 % to investigate the effects of DO levels on L-tryptophan production. Stage control of DO according to the results obtained at different DO levels was then developed for application to the production of L-tryptophan.

Analysis of fermentation products

The dry cell weight (DCW) and concentration of L-tryptophan were determined as previously described (Wang et al. 2013b). The concentrations of glucose and glutamate were monitored using an SBA-40C biosensor (Biology Institute of Shandong Academy of Sciences, Jinan, China). The acetate concentration was determined by high-performance liquid chromatography using an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with an Aminex HPX-87H HPLC column (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and refractive index detectors (RID). The injection volume of culture sample was 20 μL, and a mobile phase (flow rate 0.60 mL/min) using a solution of 0.004 mol/L H_2SO_4 was applied to the column. The column was operated at 35 °C, with a detection wavelength of 210 nm.

Analysis of metabolic flux

The distribution of metabolic flux with different DO control strategies during L-tryptophan fermentation was calculated by MATLAB (MathWorks, Natick, MA, USA) based on the analysis of metabolic flux balance and stoichiometry (Schmid et al. 2004; Huang et al. 2011).

Statistical analysis

Statistical analyses were conducted according to Wang et al. (2013b). A value of $p<0.05$ was considered to indicate statistical significance.

Results

L-Tryptophan production at different DO levels

DCW and production of L-tryptophan

The DCW and production of L-tryptophan at different DO levels (5, 20, 30, and 50 %) during L-tryptophan fermentation are presented in Fig. 1, along with the cell growth rate and L-tryptophan production rate. The lowest DCW (30.7 g/L) was obtained at a DO level of 5 %, while the DCW values at 20 and 50 % were 49.2 and 44.7 g/L, respectively. The highest DCW (50.3 g/L) was obtained at a DO level of 30 %, whereas the highest production of L-tryptophan (43.2 g/L) was obtained at a DO of 20 %. The concentrations of L-tryptophan at DO levels of 5, 30, and 50 % were 7.6, 41.3, and 35.2 g/L, respectively. The cell growth rate at a DO level of 50 % was lowest during the initial fermentation period, while the growth rate of cells at a DO level of 5 % was lowest from 10 to 38 h. The production rate of L-tryptophan at a DO level of 5 % was lowest throughout the fermentation period, while the highest production rate of L-tryptophan during the initial fermentation phase was obtained at a DO level of 20 % and the highest production rate of L-tryptophan during the later fermentation phase was obtained at a DO level of 30 %.

Concentrations of acetate and glutamate

Figure 2 shows the concentrations of acetate and glutamate and the acetate production rate at different DO levels during L-tryptophan fermentation. The highest accumulation of acetate (12.88 g/L) was obtained at a DO level of 5 %, while the lowest acetate excretion (0.86 g/L) was obtained at a DO level of 50 %. The accumulation of acetate at DO levels of 20 and 30 % was 1.32 and 1.13 g/L, respectively. The rate of acetate production was highest at a DO level of 5 %. The acetate production rate decreased with increasing DO level, while its consumption rate increased. At DO levels below 30 %, the concentration of glutamate was low during the early fermentation period (0–20 h); however, there were no significant differences in the concentration of glutamate among different DO levels. The accumulation of glutamate (11.57 g/L) obtained at a DO level of 50 % was highest; the concentrations of glutamate accumulated at DO levels of 5, 20, and 30 % were 0.47, 4.28, and 3.72 g/L, respectively.

Glucose concentration, consumption rate and conversion percentage

The glucose concentration, consumption rate and conversion percentage at different DO levels during L-tryptophan

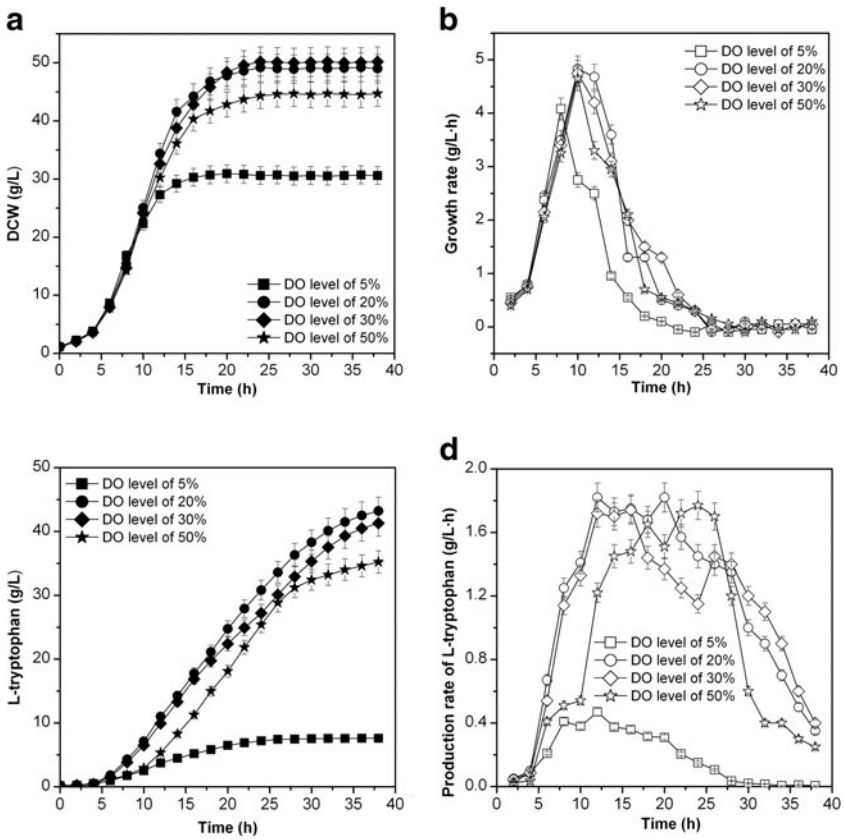


Fig. 1 Effects of DO levels on DCW and production of L-tryptophan during L-tryptophan fermentation. The DCW and growth rate of the strain obtained at DO levels of 20 and 30 % show no statistical difference, while the other data show a statistical difference ($p<0.05$). **a**, **b**, **c** and **d** DCW, growth rate of strain, production of L-tryptophan, and production rate of L-tryptophan with different DO levels, respectively

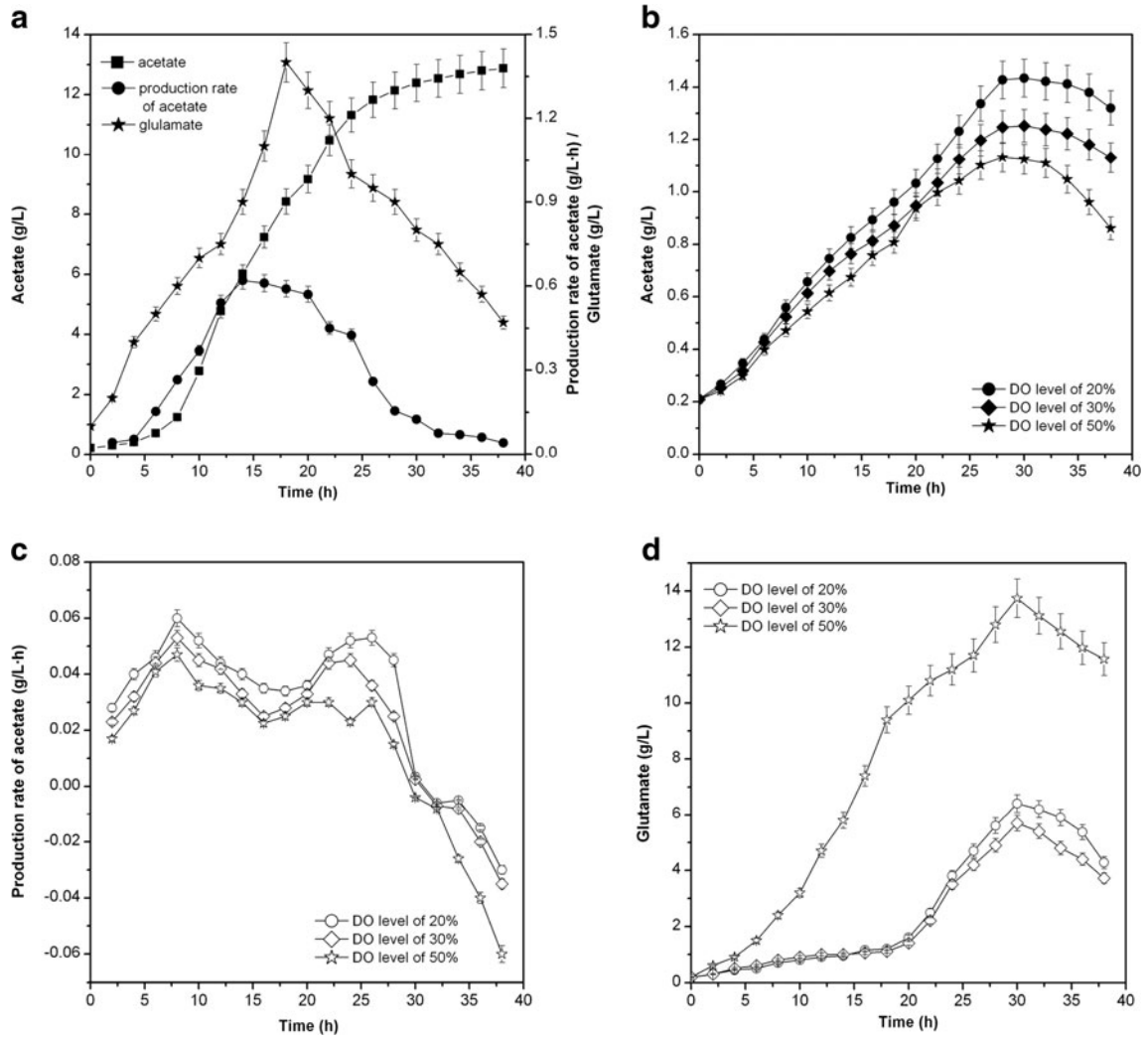
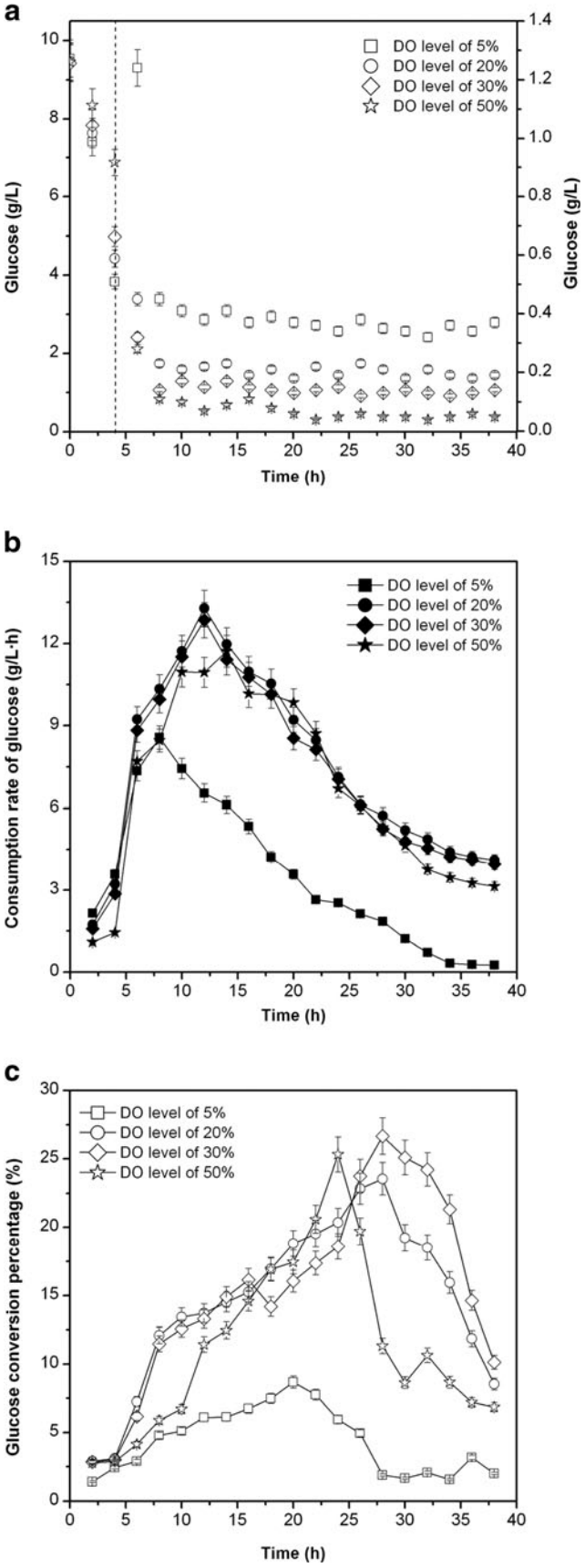


Fig. 2 Effects of DO level on concentrations of acetate and glutamate in L-tryptophan fermentation. **a** Acetate and glutamate concentrations and acetate production rate obtained at DO level of 5 %. **b**, **c** and **d** Concentration of acetate, production rate of acetate, and concentration of glutamate with DO levels of 20, 30 and 50, respectively ($p<0.05$)

fermentation are shown in Fig. 3. After the initial glucose was depleted, the glucose solution was fed into the fermenter to provide a carbon source and to control the DO level, and the concentration of residual glucose decreased with an increasing DO level. The residual glucose concentration with a DO level of 5 % was approximately 0.35 g/L, while it was below 0.10 g/L at a DO level of 50 %. The consumption of glucose increased with cell growth during the initial fermentation period, then decreased during the later period. During the initial fermentation phase, the rate of glucose consumption was highest at a DO level of 20 %, while the consumption rate at a DO level of 30 % was higher than that at 50 %. The glucose consumption rate was lowest at a DO level of 5 % at 10–38 h. During the initial fermentation phase, the glucose conversion percentage was low because glucose was primarily used for cell synthesis. In the stationary phase, glucose was primarily used for L-tryptophan biosynthesis, and glucose was converted at a higher rate. Late in fermentation, the percentage of glucose conversion decreased. The glucose conversion percentage at a DO of 5 % was lowest throughout the fermentation period, while the highest glucose conversion percentage during the later fermentation phase was obtained at a DO level of 30 %. The total glucose conversion percentages at DO levels of 5, 20, 30, and 50 % were 8.1, 16.1, 16.5, and 14.7 %, respectively.

L-tryptophan production with a DO stage control strategy

Based on the results of this study, the following four strategies of DO stage control were developed: stage I, DO 20 % (0–20 h) and DO 30 % (20–38 h); stage II, DO 30 % (0–20 h) and DO 20 % (20–38 h); stage III, DO 50 % (0–20 h) and DO 20 % (20–8 h); stage IV, DO 50 % (0–20 h) and DO 30 % (20–38 h).



◀ **Fig. 3** Effects of DO levels on glucose consumption and conversion rate in L-tryptophan fermentation. **a** Concentration of glucose with different DO levels, with the values in the left *y*-axis representing the fermentation period of 0–4 h, and the values in the right *y*-axis representing the fermentation period of 6–38 h. **b** and **c** Consumption rate of glucose and glucose conversion percentage with different DO levels, respectively ($p<0.05$)

DCW and production of L-tryptophan

Figure 4 shows the DCW and cell growth rate and the L-tryptophan production rates under different DO stage control strategies during L-tryptophan fermentation. The highest DCW (51.2 g/L) and production of L-tryptophan (46.8 g/L) were obtained during DO stage I, while the lowest DCW (46.5 g/L) and production of L-tryptophan (36.5 g/L) were obtained at DO stage III. The DCW and production of L-tryptophan under DO stage II were 50.1 and 40.3 g/L, which were 1.06 and 1.04 times as high, respectively, as those at DO stage IV. Both the cell growth rate and L-tryptophan production rate during the initial fermentation period were lower during DO stages III and IV. During the later fermentation phase, the production rate of L-tryptophan was higher at DO stages I and IV.

Concentrations of acetate and glutamate

The concentrations of acetate and glutamate under different DO stage control strategies are displayed in Fig. 5. The concentration of acetate accumulated under DO stage II (1.23 g/L) was highest, while the lowest accumulation of acetate (0.98 g/L) was obtained in DO stage IV. During the early fermentation period, the concentration and production rate of acetate decreased with higher DO levels, while the rate of acetate consumption during the later fermentation phase increased with increasing DO levels. The accumulation of acetate in DO stages I and II was 1.19 and 1.07 g/L, respectively. The concentrations of glutamate in DO stages III and IV were higher than those in DO stages I and II. The accumulation of glutamate in DO stages I, II, III, and IV was 3.28, 4.23, 6.32, and 5.79 g/L, respectively.

Glucose concentration, consumption rate and conversion percentage

The concentration, consumption rate and conversion percentage of glucose with different DO stage control strategies are presented in Fig. 6. The concentration of residual glucose was lower with higher DO levels controlled in different DO stage control strategies. The glucose consumption rates at DO stages I and II during the early fermentation phase were higher than those at DO stages III and IV. During the later fermentation period, a

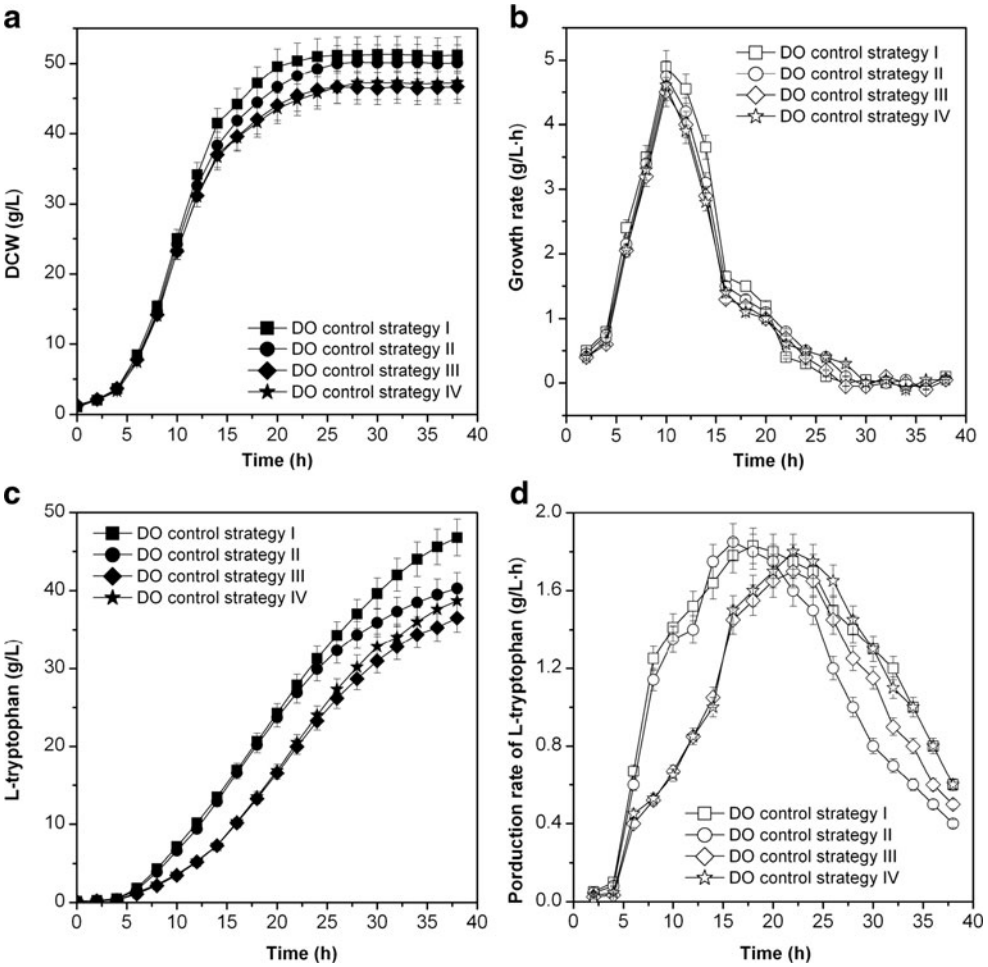


Fig. 4 Effects of DO stage control strategy on DCW and production of L-tryptophan in L-tryptophan fermentation. The difference in DCW and growth rate of the strain between DO stage control strategies I and II and between stage control strategies III and IV are not statistically significant, whereas the other data show a statistical difference ($p<0.05$). **a**, **b**, **c** and **d** DCW, growth rate of strain, production of L-tryptophan, and production rate of L-tryptophan with different DO stage control strategies, respectively

higher glucose conversion percentage was obtained during DO stages I and III. The highest glucose conversion percentage was obtained at DO stage I (17.2 %), while the glucose conversion percentages at DO stages II, III, and IV were 16.2, 15.2, and 15.8 %, respectively.

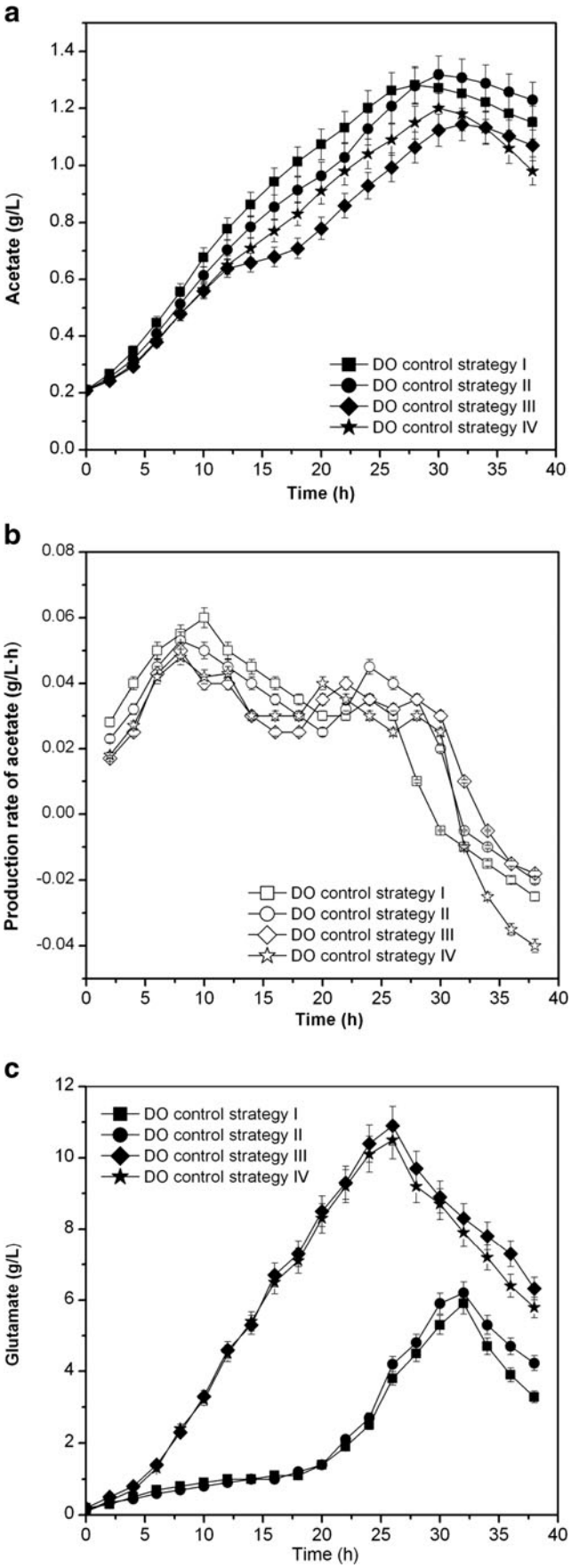
Distribution of metabolic flux with different DO stage control strategies

The metabolic flux distribution of tryptophan biosynthesis at DO stages I and III in the later fermentation period by MATLAB are presented in Fig. 7. The flux of carbon was redirected from EMP to HMP in DO stage I. Overall, 86.3 and 13.7 % of the carbon was consumed by the EMP and HMP pathways, respectively, in DO stage III, while 75.4 and 24.6 % of the carbon entered the EMP and HMP pathways during DO stage I. In DO stage I, the flux of acetate decreased from 18.9 to 4.7 %, while that of lactate decreased from 2.3 to 1.3 % and that of glutamate decreased from 4.5 to 2.4 %. The flux of tryptophan was 19.3 % in DO stage I, which was 1.79 times as high as that in DO stage III.

Discussion

DCW and production of L-tryptophan

The biomass and production of the desired product increased in response to a reduction in acetate (Eiteman and Altman 2006). During production of L-tryptophan by *E. coli*, the DCW and production of L-tryptophan were improved, while acetate accumulation decreased in response to optimization of culture conditions and genetic modifications of the strain (Cheng et al. 2012, 2013; Wang et al. 2013b). The lowest DCW and production of L-tryptophan were obtained at a DO level of 5 %, owing to the high accumulation of acetate. The lowest level of acetate was accumulated at a DO level of 50 %, but the DCW and production of L-tryptophan obtained at a DO level of 50 % were not the highest. The ROS caused by high levels of DO are potentially harmful to cells, causing damage by inactivation of proteins; however, cells possess numerous mechanisms to repair this type of damage and adapt to oxidative stress (Imlay 2008). When the DO level was maintained at 50 %, the cell growth rate was lower. At high DO levels, the flux of the HMP pathway is increased and more

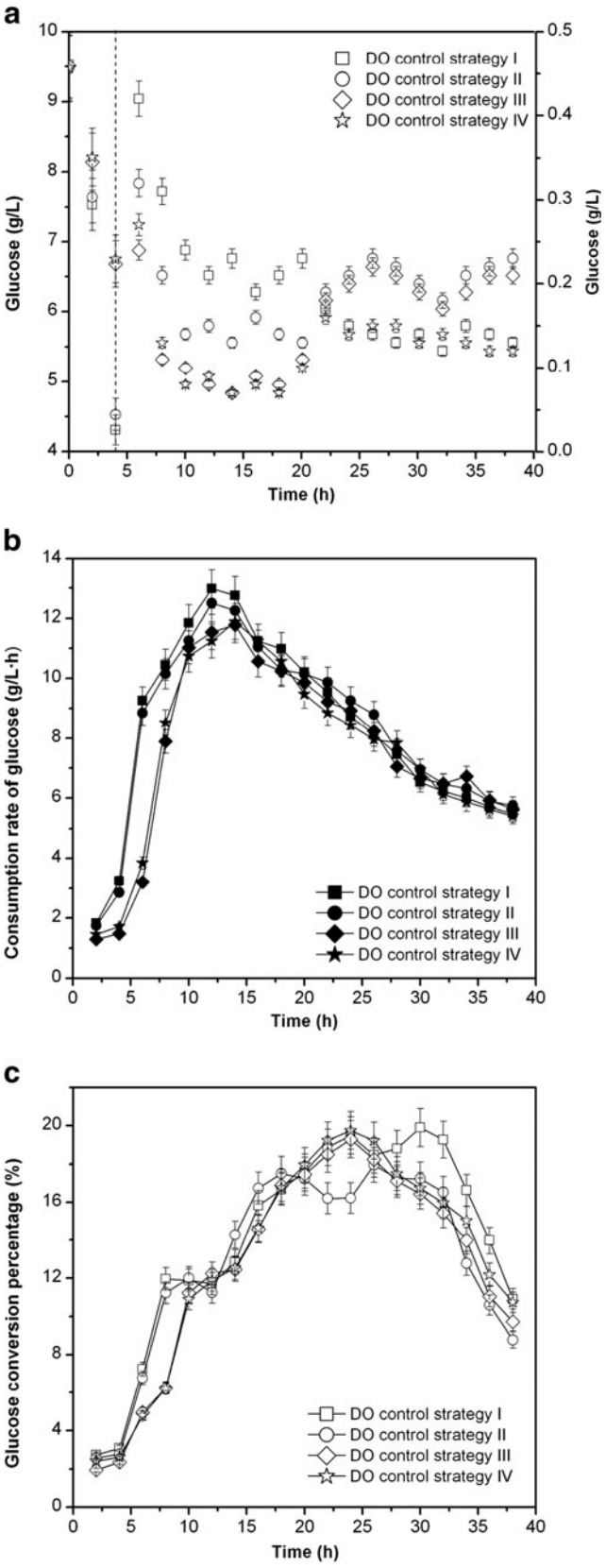


◀ **Fig. 5** Effects of DO stage control strategy on concentrations of acetate and glutamate in L-tryptophan fermentation. **a**, **b** and **c** Concentration of acetate, production rate of acetate, and concentration of glutamate with different DO stage control strategies, respectively ($p<0.05$)

NADHP is produced, which can increase the supply of E4P and exert a positive impact on the biosynthesis of aromatic compounds (Báez-Viveros et al. 2004). Moreover, the transcription of gluconeogenesis genes increases with high DO levels, leading to enhanced availability of PEP (Chandran et al. 2003; Phue and Shiloach 2005). During L-tryptophan fermentation using a DO stage control strategy, a higher L-tryptophan production rate was obtained at high DO levels. Both the TCA cycle and acetate accumulation can be affected by the transition from an unlimited to a limited oxygen supply, thereby altering cellular metabolism and protein production capabilities (Phue and Shiloach 2005). The production of L-tryptophan obtained with a DO level of 20 % was higher than that at DO levels of 5, 30 and 50 %. The concentration of glucose maintained at a low level can reduce the accumulation of acetate and increase production of L-tryptophan (Cheng et al. 2012). With regard to the DO stage control strategy, owing to the low accumulation of acetate and glutamate and the improvement in the availability of precursors for tryptophan biosynthesis when the DO level was controlled at 20 % (0–20 h) and 30 % (20–38 h), the highest DCW and production of L-tryptophan were obtained using DO stage I (Shen et al. 2012).

Concentrations of acetate and glutamate

By adjusting the feeding rate of glucose in fed-batch fermentation processes, the concentration of glucose can be maintained below a certain critical value of acetate formation, leading to a reduction in acetate excretion (Eiteman and Altman 2006; Cheng et al. 2012). The concentration of glucose was low with a high DO level controlled by adjusting the glucose feeding rate, resulting in low accumulation of acetate. Researchers have reported that the transcription levels of the gluconeogenesis (*pckA*, *ppsA*) and anaplerotic pathway (*ppc*, *sfcA*) genes are lower at low DO levels than at high levels of DO, contributing to the accumulation of pyruvate and acetyl-CoA, and causing higher accumulation of acetate through the Pta-AckA and PoxB pathways (Phue and Shiloach 2005). With high DO levels, the high transcription levels of gluconeogenic genes increase the conversion of pyruvate to glucose by gluconeogenesis, reducing the concentration of pyruvate, and consequently reducing the accumulation of acetate (Eiteman and Altman 2006). The lower transcription levels of acetyl-CoA synthetase (Acs) indicate lower acetate uptake, and the overexpression of Acs in *E. coli* results in a significant decrease in acetate accumulation and more efficient acetate assimilation (Lin et al. 2006; Báez-Viveros



◀ **Fig. 6** Effects of DO stage control strategy on glucose consumption and conversion rate in L-tryptophan fermentation. **a** Concentration of glucose with different DO stage control strategies, with values in the left *y*-axis representing the fermentation period of 0–4 h, and the values in the right *y*-axis representing the fermentation period of 6–38 h. **b** and **c** Consumption rate of glucose and glucose conversion percentage with different DO stage control strategies, respectively ($p<0.05$)

et al. 2007). During production of L-tryptophan under high DO levels, less acetate is accumulated, which is caused by the low concentration of glucose, lower acetate production, higher acetate consumption, and the variation in transcription level of relative genes for acetate formation.

Overproduction of glutamate is a significant problem during the production of tryptophan, and the reduction of glutamate formation results in higher tryptophan production performance. A previous study showed that the biosynthetic demands for NADPH were met by allowing cells to excrete glutamate, and overproduction of glutamate was caused by reducing the power imbalance (Dodge and Gerstner 2002). The coupling of NADPH production by isocitrate dehydrogenase and consumption by glutamate dehydrogenase plays an important role in glutamate production (Marx et al. 1999). The production of glutamate is also affected by the glucose feeding rate, with higher accumulation of glutamate concentrations at a higher glucose feeding rate (Dodge and Gerstner 2002). Because the DO level was controlled by adjusting the glucose feeding rate, the feeding rate of glucose decreased as the DO level increased, and the accumulation of glutamate at 20 % DO was higher than that at 30 %. Under conditions of superoxide stress, increased accumulation of α-ketoglutarate as a key precursor for glutamate biosynthesis was observed (Rittmann et al. 2003; Rui et al. 2010), which was the reason for the higher concentration of glutamate accumulated at a DO level of 50 % during L-tryptophan fermentation. However, in another study, no significant differences in the growth parameters of *E. coli* MG1655 were observed with an increase in DO level from 30 to 300 %, with the exception of a temporary decrease in the respiration and acetate accumulation profile because of the activity of the SoxRS regulon (Baez and Shiloach 2013). It may be speculated that the strain for producing L-tryptophan has a low tolerance capacity for high DO levels.

Glucose concentration, consumption rate and conversion percentage

When the DO level was controlled by adjusting the feeding rate of glucose solution, the glucose feeding rate decreased with increasing DO level, which resulted in a low concentration of glucose at a high DO level. The high concentration of ATP produced at high DO levels inhibited the activity of phosphofructokinase (PFK), leading to a reduction in the glucose consumption rate (Phue and Shiloach 2005). During L-tryptophan fermentation, the glucose consumption rate

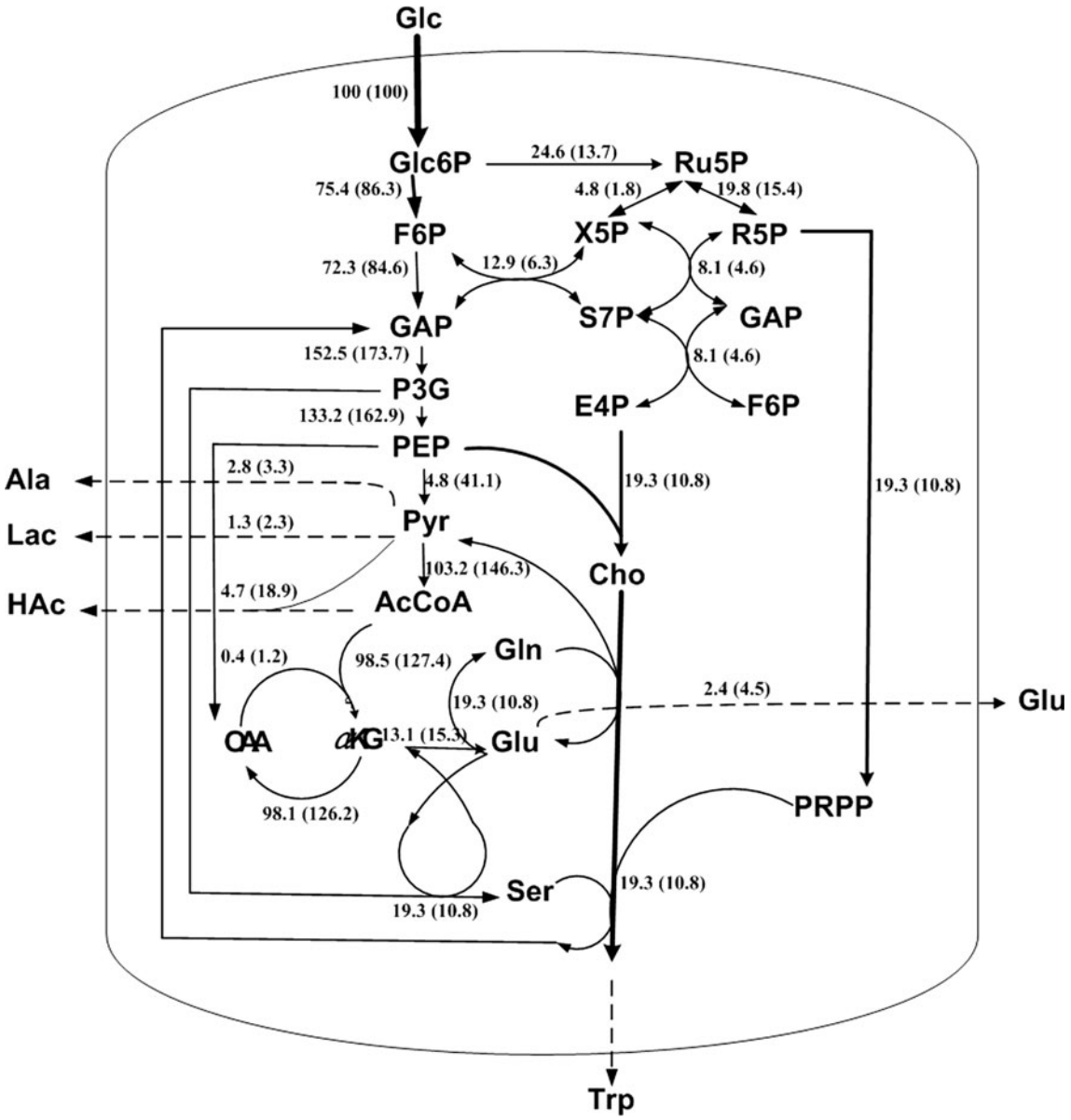


Fig. 7 Metabolic flux distribution of L-tryptophan biosynthesis with DO control stages I and III during the later fermentation period of L-tryptophan production. *Values in brackets* indicate metabolic flux in DO stage III. *Metabolite abbreviations*: *Glc* gGlucose, *GAP* glyceraldehyde-3-phosphate, *P3G* 3-phosphoglycerate, *PEP* phosphoenolpyruvate, *Pyr* pyruvate, *AcCoA* acetyl coenzyme A, *Ru5P* ribulose-5-phosphate, *X5P* xylulose-5-phosphate, *S7P* sedoheptulose-7-phosphate, *E4P* erythrose-4-phosphate, *Cho* chorismate, *PRPP* 5-phosphoribosyl pyrophosphate, *OAA* oxaloacetate, *αKG* α-ketoglutarate, *Gln* glutamine, *Glu* glutamate, *Ala* alanine, *Lac* lactate, *HAc* acetate, *Ser* serine, *Trp* tryptophan

decreased as the DO level increased, and the consumption of glucose at a DO level of 5 % was lowest because of the high accumulation of acetate. As a result of the high acetate concentration, the cell growth rate and production of the desired product were lower, which resulted in lower glucose consumption (Cheng et al. 2012). Both the deficiency in carbon use and the need for cofactor recycling to sustain balanced growth and cellular homeostasis were improved with a reduction in acetate, resulting in improved glucose and energy use rates (San et al. 2002). The overexpression of *acs* reincorporates byproducts (acetate) into the central metabolism, and then channels them into the pathway of desired production (Báez-Viveros et al. 2007). Because of the lower concentrations of acetate and glutamate and the higher rate of glucose uptake and production of L-tryptophan, the highest total glucose conversion percentage was obtained in DO stage III.

Distribution of metabolic flux

Studies have shown that the transcription levels of genes in the metabolic network and the distribution of metabolic flux are

affected by the DO level (Thomas et al. 1972; Phue and Shiloach 2005). The lower activity of PFK and glucose consumption with higher DO was found to result in less carbon flux into the EMP pathway (Phue and Shiloach 2005; Rui et al. 2010). The flux of pyruvate with high DO levels decreased due to a reduction in the EMP pathway flux and improved gluconeogenesis, which led to a reduction in acetate and lactate flux (Castaño-Cerezo et al. 2009). The production of lactate has been reported to be caused by pyruvate accumulation (Zhu and Shimizu 2005). Because of the high glucose feeding rate during DO stage III, the accumulation and flux of glutamate were found to increase (Dodge and Gerstner 2002). At DO stage I, the rate of L-tryptophan production was higher because of the redistribution of metabolic flux and improved availability of precursors, which was in accordance with the higher flux of tryptophan synthesis observed upon analysis of the metabolic flux distribution.

In summary, in the present study, the impact of DO level on the production of L-tryptophan was investigated based on the transcription levels of genes related to the formation of acetate and biosynthesis of tryptophan. In addition, a DO feedback feeding strategy was used to control DO level to remedy the insufficient oxygen provided by the fermenter. Higher production of L-tryptophan and glucose conversion percentage were obtained with the DO level controlled at 20 % (0–20 h) and 30 % (20–38 h) as a result of the lower accumulation of byproducts and higher flux for tryptophan biosynthesis. This study illustrates a useful approach for large-scale production of L-tryptophan, with increased L-tryptophan yields and decreased production costs, which can expand the market for L-tryptophan and widen the application of L-tryptophan as a supplement of animal feed. The DO control strategy applied in the present study can be used in the production of other amino acids, thus maintaining the DO level and glucose concentration at certain values and avoiding the issue of insufficient oxygen supply provided by the fermenter.

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