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Fed-batch Self-regulated Fermentation of Glucose to Co-produce Glycerol and 1,3-propanediol by Recombinant *Escherichia coli*

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ABSTRACT: As important bio-chemicals, glycerol and 1,3-propanediol (1,3-PDO) have been widely used in numerous fields, e.g., polymers, cosmetics, foods, lubricants, medicines, and so on. Bio-based 1,3-PDO is generally produced from glycerol or glucose by natural or recombinant strains, during which organic acids are always co-produced. In this work, acetic acid was also co-produced when 1,3-PDO was obtained from glucose by a recombinant strain of *E. coli* MG1655. Usually, a base was added to adjust the fermentation pH, resulting in the accumulation of organic salts and difficulty in the next down streaming process. Herein, a novel strategy was developed to consume the produced acetic acid by cells self in fed-batch self-regulated fermentation. The recombinant *E. coli* cells produced 48.33 g/L glycerol and 61.27 g/L 1,3-PDO with a total mass yield of 45.6% and without any other byproducts at the end of 5 fed-batch fermentations. The initial buffer pH, glucose concentration, pulse feeding sugar amount, time for a single batch fermentation and reducing acid were investigated by a series of comparative experiments. This fed-batch self-regulated fermentation has potential for the co-production of 1,3-PDO and glycerol, and will highlight the subsequent modification of recombinant *E. coli* strain by synthetic biology.

Keywords: 1,3-propanediol, Glycerol, Acetic acid, Self-regulated fermentation, Recombinant *E. coli*

1. Introduction

As one of the important bulk chemicals, 1,3-propanediol (1,3-PDO) is widely used as a raw material in many fields, such as plastics, calming agents, fungicides, detergents, cosmetics and so on. In particular, it is the key synthetic monomer for the new polyester, polypropylene terephthalate (PTT). PTT has outstanding overall performance with greater advantages than polyethylene terephthalate (PET) and polybutylene terephthalate (PBT) in terms of softness, elasticity, stain resistance and iron-free properties [1–3], and will have an increasing demand in the textile industry in the future. With the rapid development of textile industry, the demand of 1,3-PDO is also increasing rapidly.

Glycerol is a common substrate for the production of 1,3-PDOby natural microorganisms, in which glycerol metabolism is divided into oxidation and reduction branches. Generally, glycerol is oxidized in multiple steps to produce pyruvate and by-products, such as acetate, butyrate, formate, 2,3-butanediol, ethanol, etc. [4]. The ATP and NADH produced by the oxidation branch are used for cell growth metabolism as well as synthesis of 1,3-PDO in reduction branch. Therefore, the production of 1,3-propanediol from glycerol by natural microorganisms is necessarily coupled with the production of various oxidative by-products [5]. The most prominent natural microorganisms are *Klebsiella* [6,7] and *Clostridium butyricum* (*C. butyricum*) [8], but *Klebsiella* is pathogenic and produces 2,3-butanediol, which is very similar to 1,3-PDO, as a by-product. The growth of *C. butyricum* requires an anaerobic environment and coproduces acetate and butyrate [9]. The presence of by-products creates a huge economic burden for 1,3-PDO separation. In the past years, there has been a great effort to reduce the formation of by-products in order to increase the product yield and simplify the downstream processing.

Although many metabolic engineering approaches have been employed to decrease the generation of these byproducts by inactivating their biosynthetic pathways [10,11], a large number of unwanted byproducts, especially organic acids, are still produced. Several metabolic engineering operations were performed to eliminate the by-product acetic acid by overexpressing heterologous

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acetyl coenzyme A synthetase, enhancing the acetic acid assimilation pathway, and further reprogramming intracellular carbon metabolism [12], but acetic acid was still present. For the production of 1,3-PDO, the accumulation of organic acids lowered the fermentation pH and inhibited the growth of microbial cells, resulting in extra consumption of base to adjust pH and a burden on the downstream processing for separation of 1,3-PDO from organic salts.

In recent years, the development and application of self-regulated fermentation has attracted extensive attention in the field of biosynthesis, such as the design and construction of a self-regulating enzyme-based fed-batch fermentation [13], as well as stable and self-regulated microbial consortia for maintaining pH, redox and thermodynamic potential in the reactor [5]. An interesting phenomenon is that the cellular self-regulation can be used for detoxification or disinhibition on cell growth. For example, CHO cell line can uptake the extracellular lactate produced from glucose metabolism so as to change the culture conditions from an inhibitory to a suitable environment [14–16]. That is detoxification by pH self-regulation. In contrast to the aforementioned metabolic engineering approaches, self-regulated fermentation offers the possibility of disappearance of organic acids produced as by-products during microbial production of 1,3-PDO.

In this work, the structural gene *yqhD* encoding 1,3-PDO oxidoreductase isoenzyme from *Escherichia coli* (*E. coli*) and gene *dhaB* encoding glycerol dehydratase from *Klebsiella pneumoniae* were upward expressed in a novel recombinant strain *E. coli* MG1655 (pSC101_PDO). In addition, we modified the glucose transport system and the central carbon metabolism of MG1655 strain, and also deleted some genes encoding the glycerol oxidation pathway, so that more carbon sources can flow to the pathway of 1,3-PDO synthesis. The optimization of fermentation conditions for improving 1,3-PDO production by recombinant *E. coli* is becoming one of the major goals in biotechnology today, although previous attention has principally been paid to the regulation of gene expression. Herein, a new strategy was developed to address the difficulties of the downstream processing caused by the accumulation of organic salts with a fed-batch self-regulated fermentation. This fermentation mode has the potential to co-produce 1,3-PDO and glycerol and will be constructive for subsequent modifications of recombinant *E. coli* strains.

2. Materials and Methods

2.1. Materials

Standards of glucose, 1,3-PDO, glycerol and acetic acid were purchased from Sigma-Aldrich (Saint Louis, MO, USA). All other chemicals used in this study were analytical grade and purchased from Aladdin (Shanghai, China).

2.2. Microbial Strains and Plasmids

Table 1 shows microbial strains and plasmids used in this study. *E. coli* MG1655 was used as a parental strain for engineering its metabolic pathway. *E. coli* DH5α was used to maintain constructed plasmids.

Table 1. Strains and plasmids used for this study.

Strains and Plasmids	Relevant Characteristics
Strains	
E. coli DH5α	Standard cloning strain
E. coli MG1655	$F^- \lambda^-$ *ilvG*$^-$ *rfb-50 rph-1*
FC12	*E. coli* MG1655 Δ*glpK* Δ*gldA* Δ*ptsHIcrr* Δ*ndh* P_{trc}-*galP* Δ*arcA* Δ*edd* $P_{GI1.5}$-*gapA* $P_{GI1.6}$-*ppc* Δ*mgsA* $P_{GI1.6}$-*yqhD*, harboring pSC101_PDO
Plasmids	
pSC101_TIMER	pSC101 replicon, P_{ybaJ}-timer^{bac}, Kan^R
pSC101_PDO	pSC101 replicon, Kan^R, expressing GPD1 and GPP2 from *Saccharomycses cerevisiae* under the control of *trc* promoter, and *dhaB* genes from *Klebsiella pneumoniae* under the control of *GI1.6* promoter
pREDCas9	$pSC101^{ts}$, P_{Lac}-λ-Red, Cas9, P_{araBAD}-gRNA-bla, Spc^R
pGRB	pUC18 for gRNA construction

CRISPR-Cas9 mediated genome editing of *E. coli* MG1655 was carried out using two plasmids pREDCas9 and pGRB as described previously [17]. The sgRNAs was selected from previous studies [18] or online gRNA design tool (https://www.atum.bio/eCommerce/cas9/input). The 20 bp spacer sequences were inserted into pGRB plasmid to replace the lacZ segment by using Gibson assembly [19,20] or T5 exonuclease DNA assembly [21]. Approximately 500 bp flanking sequences of each editing target were amplified from genomic DNA of *E. coli* MG1655, spliced with overlap extension PCR [22], and used as donor DNA for homologous recombination mediated double strand repair. For promoter replacement, the *trc* promoter and glucose isomerase promoter from *Streptomyces lividins* and their variants were used according to US patent No. 7,371,558. Specific promoter sequences were introduced into the 5′ end of primers and inserted into donor DNA by overlap extension PCR as well. Iterative genome editing was carried out as previously described. pSC101_PDO plasmid was introduced into the final strain with 12 chromosomal modifications and the resultant strain *E. coli* FC12 (Table 1) was used for fed-batch fermentation in shake flasks.

2.3. Media

The medium composition was similar to that described by DuPont [20]. The preculture medium contains (g/L): yeast extract 5, tryptone 10, NaCl 10. The fermentation medium contains the following components (g/L): glucose (according to the experimental needs), K_2HPO_4 13.6, KH_2PO_4 13.6, $(NH_4)_2SO_4$ 3.2, $MgSO_4{\cdot}7H_2O$ 2, ferric ammonium citrate 0.3, citric acid monohydrate 0.42, yeast extract 5, and 1 mL of trace element solution. The trace element solution contains the following components (g/L): Citric acid$\cdot H_2O$ 4.0, $MnSO_4{\cdot}H_2O$ 3.0, NaCl 1.0, $FeSO_4{\cdot}7H_2O$ 0.10, $CoCl_2{\cdot}6H_2O$ 0.10, $ZnSO_4{\cdot}7H_2O$ 0.10, $CuSO_4{\cdot}5H_2O$ 0.010, H_3BO_3 0.010 and $Na_2MoO_4{\cdot}2H_2O$ 0.010.

A certain amount of antibiotic was added to identify the recombinant *E. coli* MG1655 during the activation and fermentation stages. Kanamycin sulfate was added at 50 mg/L in this study.

2.4. Culture Conditions

Seed culture was carried out in 250 mL conical flasks containing 50 mL of seed medium sterilized at 115 °C for 15 min, and the mouth of the flask was covered with a permeable membrane for aerobic fermentation. 2% (*v*/*v*) of the strain stored at −80 °C was activated at 37 °C and 200 rpm for 12 h. After the activation culture, 2% broth was inoculated into the fermentation medium. Batch fermentation and fed-batch self-regulated fermentation were carried out in 250 mL conical flasks. During the whole fermentation process, the effects of temperature and initial pH on fermentation were investigated. The common temperatures for *E. coli* culture, e.g., 30 °C, 34 °C or 37 °C, were selected for the study. In addition, the common pH range of *E. coli* culture is 6.0–8.0 [23], so six groups of experiments were set up in this interval, namely, initial pH = 6.0, 6.4, 6.8, 7.2, 7.6, and the pH was not controlled during fermentation. 1,3-PDO and glycerol concentrations and conversion rates were measured at the end of fermentation, and the pH at the endpoint was recorded to determine the most suitable temperature and pH for the recombinant *E. coli* FC 12. 20 g/L glucose was used as the substrate for both temperature and pH experiments, and additionally, other culture conditions were identical to those of seed culture.

2.5. Batch Fermentation and Fed-batch Self-regulated Fermentation

Batch and fed-batch self-regulated fermentations were performed in 250 mL conical flasks with 50 ml working volume and 2% (*v*/*v*) inoculum. The batch fermentations were carried out at 34 °C, pH 6.8, 200 rpm, and glucose concentrations of 20, 30, 40, 50, 60, and 70 g/L.

The fed-batch self-regulated fermentation was a kind of fed-batch fermentation, during which the substrate (glucose) was added when acetic acid produced in a batch fermentation was consumed by the bacterium itself without adding a base to recover the original pH. The fed-batch self-regulated fermentations were also conducted at 34 °C, pH 6.8, and 200 rpm. The initial glucose concentration was 20 g/L or 50 g/L. The substrate glucose was added into flasks at 20 or 50 g/L when glucose was completely consumed and the pH returned to 6.8 again at each batch fermentation. When the *E. coli* FC12 stopped growing, no more glucose was added.

In order to investigate utilization of intermetabolites in *E. coli* FC 12, exogenous glycerol and acetic acid were added after a batch fermentation at 20 g/L glucose. When the pH first dropped and then recovered to 6.8, a certain amount of acetic acid was added manually to make the substrate pH drop to 6.0–6.4. Meanwhile, 1–2 g/L glycerol was added into flasks. Once the pH recovered to 6.8, the operation was repeated until *E. coli* FC12 stopped growing.

2.6. Analytical Methods

The optical density of the cultured broth was measured at 600 nm using a UV-Vis spectroscopy system. Glucose concentration was determined by saccharimeter. The concentrations of glycerol, 1,3-PDO, acetate, lactate, xylose, arabinose and cellobiose were determined by a high-performance liquid chromatography (HPLC) analytical system (water 1515) using an Amino HPX 87H column (300 mm × 7.8 mm; Bio-Rad, Hercules, CA, USA) combined with an autosampler (water 2707) and a differential refractometer (water 2414). The HPLC operating conditions were as follows: sample volume of 20 μL, mobile phase 5 mmol/L H_2SO_4, flow rate 0.6 mL/min, detector temperature 35 °C, column temperature 65 °C [24]. The test samples were obtained from fermentation broth after centrifugation at 12000 r/min for 10 min, and were properly diluted before the test and filtered through a 0.22 μm membrane filter.

2.7. Statistical Analysis

Linear regression was accomplished using Microsoft Excel software after collecting experimental data. A significance test was recommended using two-tailed paired Student's *t* test, and $p < 0.05$ was considered to be significant.

3. Results and Discussion

3.1. Culture Conditions for Batch Fermentations

During fermentation, the temperature and pH are the main factors that affect cell growth and product formation [25]. The effect of temperature on the fermentation of engineered bacteria is reflected in several aspects, such as affecting the physicochemical properties of the fermentation substrate, the cell growth and metabolism, and the production of target products. In addition, CO_2 and acetic acid produced during fermentation will decrease the pH value of the fermentation, which in turn affects the concentration of H^+ or OH^- in the bacterial cells as well as the activity of intracellular enzymes [26]. Therefore, it is important to select the appropriate pH value and keep it relatively stable for the fermentation of engineered bacteria [27,28].

To investigate the optimum temperature of *E. coli* FC12, the shaker culture was set at three common temperatures, i.e., 30 °C, 34 °C, and 37 °C, respectively. As shown in Figure 1, *E. coli* FC12 produced the highest concentration and conversion rate of 1,3-PDO at the same substrate concentration with 34 °C incubation. Therefore, 34 °C was selected in the subsequent experiments.

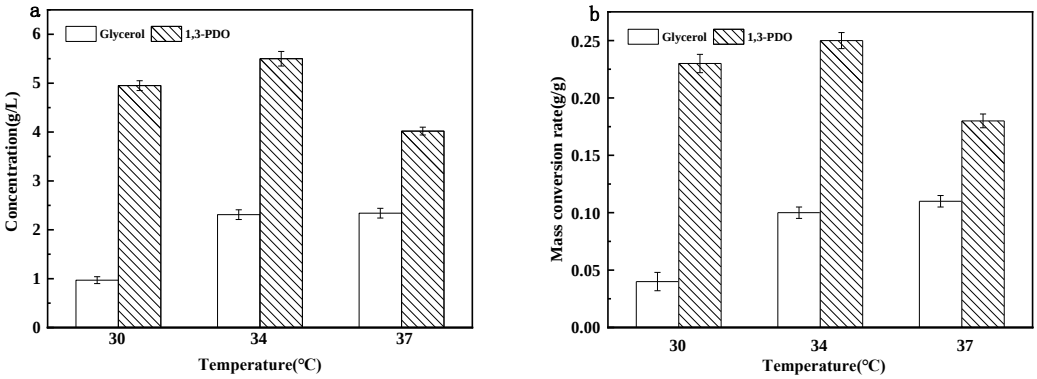

Figure 1. Comparison of fermentation product concentrations and conversion rates of *E. coli* FC12 at different temperatures. (**a**) Product concentrations at different temperatures; (**b**) Product conversion rates at different temperatures.

As previously described, six groups of experiments at different initial pH were carried out at a substrate concentration of 20 g/L glucose. As shown in Figure 2, large differences existed in the fermentation performance of *E. coli* FC 12 at different initial pHs. When the initial pH 6.0 or 8.0 was chosen, the concentration and conversion of glycerol and 1,3-PDO were low, probably due to the poor growth state of the bacterial cells caused by too low or high initial pH. If the initial pH was chosen to be 6.4–7.6, the strain showed better performance. At pH 7.2, the concentration of 1,3-PDO was 5.82 g/ L, and its mass conversion rate reached 0.27 $g_{1,3\text{-PDO}}/g_{Glucose}$, which was higher than other cases.

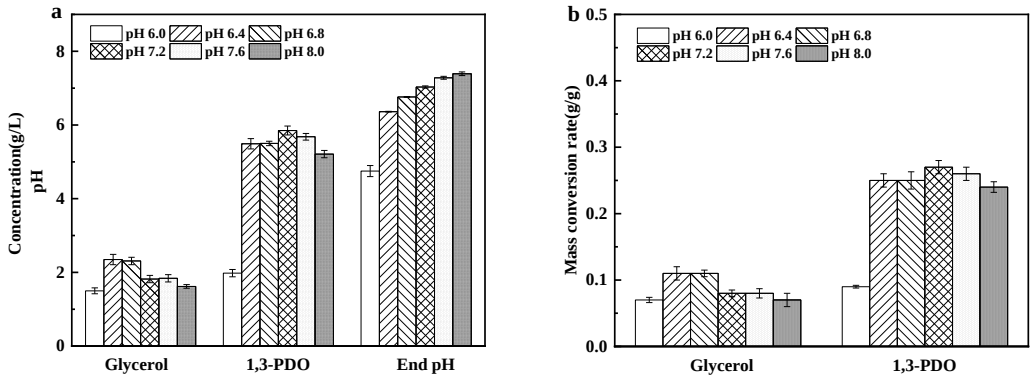

Figure 2. Fermentation results at different pH values. (**a**) Concentration of products and pH reduction; (**b**) Conversion rate of products.

The buffering capacity of the fermentation medium for organic acids was further investigated at high glucose concentration as shown in Figure 3. Unfortunately, as the substrate concentration gradually increases, the ability of the strain to consume glucose gradually decreased at the initial pH 7.2, and a large amount of $CaCO_3$ needed to be added to maintain the stable pH. When the glucose concentration was 60 g/L, the strain could not fully utilize glucose at the end of fermentation. If phosphate was added to meet microbial growth and maintain osmolarity, the pH could be regulated by the phosphate buffer system [29]. However, the ability of phosphate buffer at pH 7.2 was limited at high substrate concentrations.

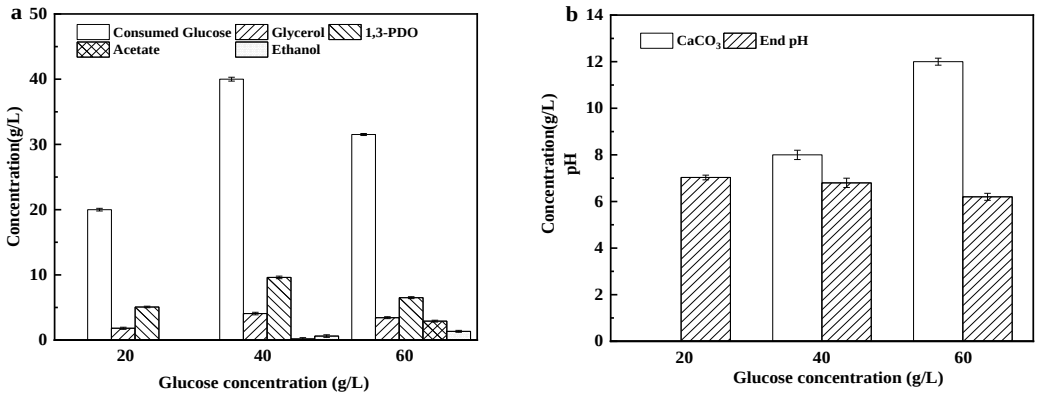

Figure 3. Fermentation performance of *E. coli* FC 12 at different glucose concentrations at pH 7.2. (**a**) Product concentration and substrate consumption; (**b**) $CaCO_3$ addition and end pH of fermentation.

Figure 4 shows almost no change in pH at the beginning and end of fermentation when the initial pH was adjusted to 6.8. It is very important that the fermentation did not require the addition of $CaCO_3$ to maintain the pH stability. Furthermore, except glycerol and 1,3-PDO, no other by-products were present in the fermentation broth when the substrate was consumed out, which was an obvious advantage for downstream separation. Meanwhile, the mass conversion of 1,3-PDO gradually decreased and the mass conversion of glycerol gradually increased with the increase of glucose concentration, and their sum increased first and then decreased. The maximum mass conversion rate of 1,3-PDO was 0.34 $g_{1,3\text{-PDO}}/g_{Glucose}$ at glucose concentration of 20 g/L, and the maximum mass conversion rate of glycerol and 1,3-PDO was 0.54 $g_{1,3\text{-PDO+glycerol}}/g_{Glucose}$ at the glucose concentration of 50 g/L.

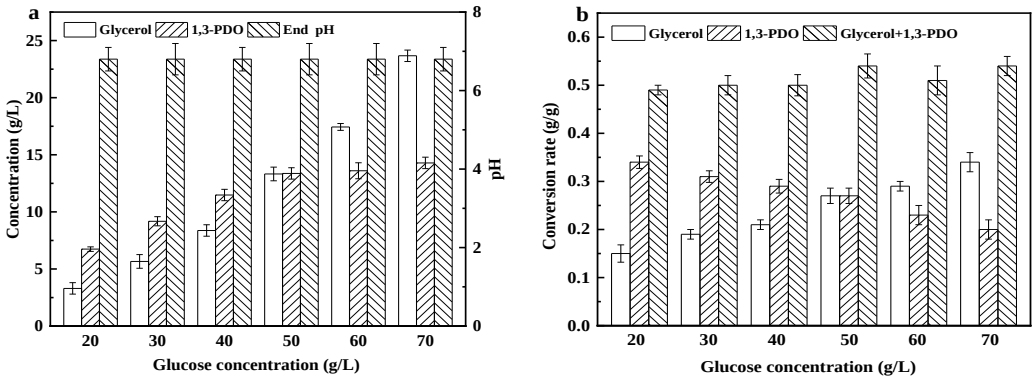

Figure 4. Fermentation performance of *E. coli* FC12 at different glucose concentrations at pH 6.8. (**a**) Product concentration and end pH of fermentation; (**b**) Glycerol, 1,3-PDO conversion rates and their sum.

During the fermentation time course as shown in Figure 5, the batch fermentation at 20 g/L glucose could be divided into two stages. At the first one, from the beginning of fermentation to 24 h, the substrate was consumed completely, accompanied by the production of glycerol and 1,3-PDO as well as acetate, leading to a pH decline. At the second stage (between 24 h and 36 h), 1,3-PDO increased slightly, glycerol decreased slightly by 1.06 g/L, and acetic acid was gradually utilized, resulting in pH recovery as the same to the starting pH. According to previous reports, pH is critical for initiating the metabolic shift from lactate production to concomitant consumption of lactate and glucose. In *HEK293* cell culture, this shift can be triggered at pH 6.8, and *E. coli* MG1655 behaves similarly [30]. Additionally, a similar situation occurred with a decrease in acetic acid accompanied by a small increase in 1,3-PDO of 0.44 g/L [31], which was very similar to the fed-batch self-regulated fermentations. The pH self-regulated fermentation provides an important way for solving the problem of acetate detoxification and avoiding carbon source waste.

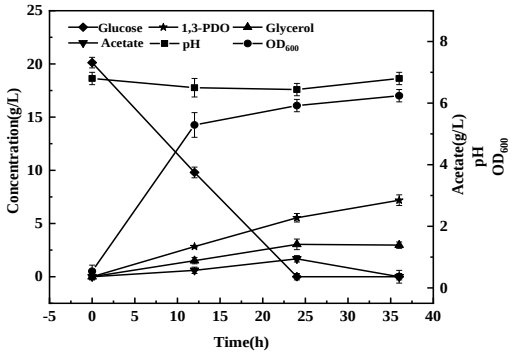

Figure 5. The shake flask fermentation at glucose concentration of 20 g/L.

3.2. Fed-batch Self-regulated Fermentation for Co-production of 1,3-PDO and Glycerol

To evaluate the ability of *E. coli* FC12 to produce glycerol and 1,3-PDO, a fed-batch self-regulated fermentation as mentioned in Section 2.5 was performed by adding glucose to 20 g/L or 50 g/L at the end of each batch fermentation. As shown in Figure 6a, acetate was produced and then disappeared in each batch fermentation, resulting in oscillation of pH from 6.8 to 6.2. As feeding glucose to 20 g/L, the glycerol concentration inclined fluctuantly, and 1,3-PDO increased continuously with the highest 1,3-PDO mass conversion rate of 0.33 $g_{1,3\text{-PDO}}/g_{Glucose}$ as shown in Figure 6b. After 248.53 g/L of glucose was fed by 12 times in 312 h, the final 1,3-PDO concentration was 60.18 g/L and the glycerol concentration was 39.69 g/L in the fermentation broth without any other by-products.

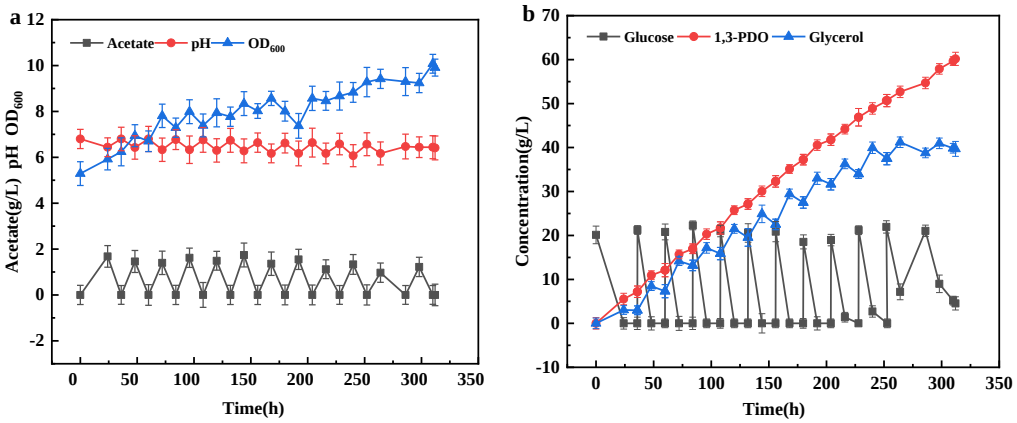

Figure 6. Time course of fed-batch self-regulated fermentation at 20g/L glucose. (**a**) Acetic acid concentration, pH and OD600; (**b**) Glucose, 1,3-PDO and glycerol concentration.

Similar results were obtained by fed-batch self-regulated fermentation at 50 g/L glucose as shown in Figure 7. When the cumulative glucose of 240.25 g/L was fed by 5 times in 300 h, the final glycerol and 1,3-PDO concentration was 48.33 and 61.27 g/L, respectively, without any other by-products.

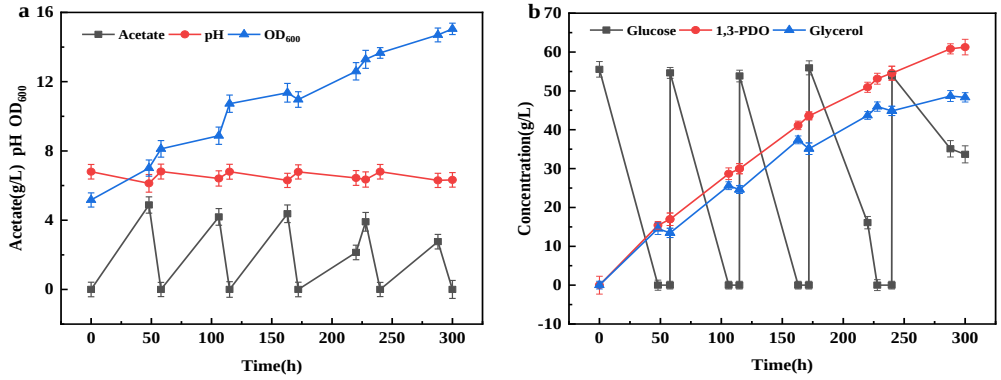

Figure 7. Time course of fed-batch self-regulated fermentation at 50 g/L glucose. (**a**) Acetic acid concentration, pH and OD_{600}; (**b**) Glucose, 1,3-PDO and glycerol concentration.

Comparison of two different feeding strategies concluded that the fed-batch self-regulated fermentation at 50 g/L glucose could receive higher concentrations of 1,3-PDO and glycerol in shorter fermentation time than those at 20 g/L glucose, resulting in higher total conversion rate (45.6% vs. 40.2%) and productivity (0.37 vs. 0.32 g/(L·h)). This was in accordance with the fact that the total mass conversion rate of glycerol and 1,3-PDO in batch fermentation at 50 g/L glucose was higher than those at 20 g/L glucose as seen in Figure 4b.

In Table 2, we summarize the yield, productivity and major byproducts of different microbial methods for the preparation of 1,3-PDO in recent years. As shown in Table 2, natural microbial strains as well as microbial consortia can achieve high concentration of 1,3-PDO, but all have different kinds of by-products, such as acetic acid, lactic acid, butyric acid, succinic acid, etc. The presence of organic acid by-products is not conducive to downstream isolation. Most genetically engineered bacteria are almost free of organic acids after modification. Compared to the engineered strain used by Dupont, *E. coli* FC12 in this study has the disadvantage of low productivity, mainly because of the long time for self-adjusting pH to consume the byproduct (acetic acid). However, fed-batch self-regulated fermentation does not require pH adjustment, saving the cost of bases and simplifying the fermentation operation process. Furthermore, to address the problem of low productivity during the fermentation of the bacterium, we will further improve the strain in the future to accelerate the acetic acid reuse pathway to further increase the productivity.

Table 2. Preparation of 1,3-PDO by microbial method.

Organnism	Mode of Operation	Titer ($g \cdot L^{-1}$)	Yield ($mol \cdot mol^{-1}$)	Overall Productivity ($g \cdot L^{-1} \cdot h^{-1}$)	Major Byproducts	Reference
Natural strains						
C. butyricum DSP1	Fed-batch	71.0	0.65	0.68	Butyrate (14–20 g/L) Lactate (16–17 g/L) Acetate (8–11 g/L)	[39]
C. butyricum AKR102a	Fed-batch	76.2	0.61	2.30	Butyrate (12.5 g/L) Lactate (6.0 g/L) Acetate (7.7 g/L)	[40]
K. pneumoniae LX3	Fed-batch	68.2	0.62	3.43	Lactate (5.09 g/L) Ethanol (7.63 g/L)	[41]
L. diolivorans DSM 14421	Fed-batch	85.4	0.56	0.46	Lactate (3.4 g/L) Acetate (6.4 g/L)	[42]
Ci. freundii VK-19	Fed-batch	40.2	0.37	0.47	Lactate (21.7 g/L) Succinate (7.3 g/L)	[43]
Microbial consortium						
Microbial consortium C2-2M	Fed-batch	82.7	0.66	3.06	Butyrate (16.85 g/L) Acetate (7.90 g/L)	[44]
Microbial consortium DL38	Batch	81.4	0.63	0.99	Lactate (7.73 g/L) Acetate (7.63 g/L)	[45]
Microbial consortium LS30	Fed-batch	27.8	0.45	0.44	Lactate (14.68 g/L) Acetate (9.05 g/L)	[46]
Genetically engineered bacterium						
Bacteria for DuPont	Fed-batch	135.0	0.46	3.50	Presence of by-products, not reported	[26]
E. coli DH5α/ pQKG, pSCD	Batch	12.1	0.40	0.20	None	[47]
Systems metabolic engineering of *Vibrio natriegens*	Fed-batch	56.2	0.61	2.36	None	[48]
This study	Self-regulated fed-batch	61.3	0.60	0.21	None	-

High concentrations of acetic acid/acetate in culture media can inhibit cell growth, reduce biomass, and lower the yield of target products. In recent years, inexpensive acetic acids have been gradually developed as raw materials for the production of high value-added products [32–34]. In this study, acetic acid detoxification was achieved by fed-batch self-regulated fermentation of *E. coli* FC12 without any by-products at the end of fermentation. However, the total fermentation time was as long as 300 h because of slow utilization of acetic acid. Undoubtedly, it is necessary to further improve acetate utilization. Some previous efforts could guide the improvement of *E. coli* FC12. For example, the consumption rate of acetate was significantly higher in engineered strains obtained by overexpressing *acs* in *E. coli* than the control strains [35]. In addition, acetate consumption could be enhanced by knocking down the negative regulators of glyoxylate cycle expression *iclR* [36] and *fadR* [37].

3.3. Fate of Intermediates in Fed-batch Self-regulated Fermentation

In the fed-batch self-regulated fermentation of 1,3-PDO as seen in Figures 6 and 7, glycerol as intermediate was not completely conversed to 1,3-PDO at the end of fermentation, and the remaining glycerol concentration was 40–50 g/L. However, acetate as by-product could be completely consumed. To explore the relationship between glycerol, acetic acid, and 1,3-PDO as well as biomass, fed-batch self-regulated fermentations were carried out by adding glycerol and acetic acid into flasks. The experiment was conducted when the biomass (OD_{600}) was greater than 6.0 at the fermentation time of 12 h.

As shown in Figure 8a, only little change of 1,3-PDO concentration could be observed despite what happened in extra supplementation glycerol and acetic acid. In the later period from 48 to 83 h, acetic acid was consumed for cell growth of *E. coli* FC12 (Figure 8b). Meanwhile, glycerol accumulated steadily. This implied that extracellular glycerol was not efficiently converted into 1,3-PDO by *E. coli* FC12. This was also the reason for accumulation of glycerol as shown in Figures 6 and 7.

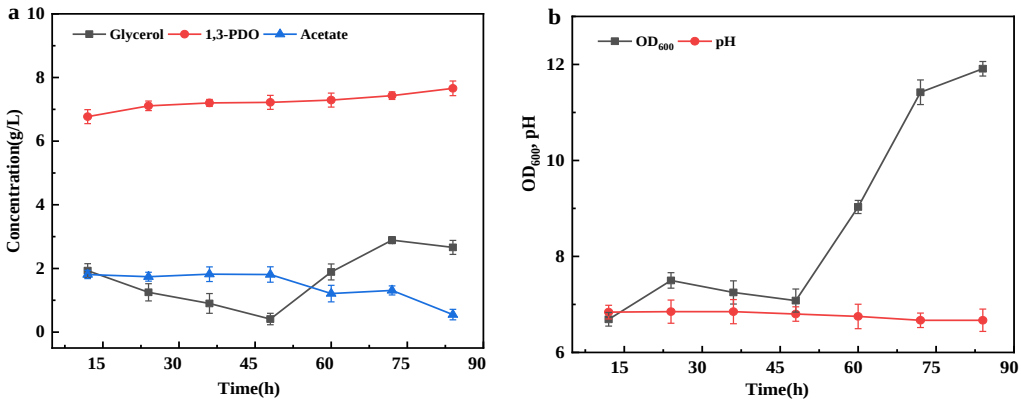

Figure 8. Time course of fed-batch self-regulated fermentations by adding glycerol and acetic acid. (**a**) 1,3-PDO, acetic acid and glycerol concentration; (**b**) pH and OD_{600}.

The above results are enlightening for the subsequent improvement of the organism: (a) to accelerate the conversion efficiency of glycerol to 1,3-PDO and to make full use of intracellular glycerol by overexpression of glycerol dehydratase and its activating protein; (b) to reduce glycerol secretion by deletion of gene encoding GlpF for glycerol facilitator. Some previous reports showed that the *glpF*-disrupted organisms could increase 5–7-fold intracellular glycerol accumulation and 2–3-fold 1,3-PDO titers [38].

4. Conclusions

In this study, a new strategy was developed to fully utilize the maximum potential of *E. coli* FC12 for coproduction of 1,3-PDO and glycerol without any other by-products. This cellular self-consumption of acetic acid in fed-batch fermentation can relieve both the cellular toxicity of acetic acid and the removal of organic salts from the fermentation broth in downstream processing, reducing the separation steps and saving fermentation and separation costs due to no addition of base for pH stability. The experimental results also provide constructive guidelines for subsequent modifications of recombinant *E. coli* strains by synthetic biology.

Author Contributions

Conceptualization: Z.L. Xiu and Z.W. Zhu; Methodology, Z.L. Xiu and Y.Q. Sun; Investigation, G.M. Liu; Strain Construction: C. Feng; Writing—Original Draft; G.M. Liu, and C. Feng; Writing—Review & Editing, Z.L. Xiu and Y.Q. Sun; Supervision, Z.L. Xiu and Z.W. Zhu.

Ethics Statement

Not applicable.

Informed Consent Statement

Not applicable.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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