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FERMENTATION, CELL CULTURE AND BIOENGINEERING - ORIGINAL PAPER

Rapid and stable production of 2,3-butanediol by an engineered *Saccharomyces cerevisiae* strain in a continuous airlift bioreactor

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Abstract
Utilization of renewable feedstocks for the production of bio-based bulk chemicals, such as 2,3-butanediol (2,3-BDO), by engineered strains of the non-pathogenic yeast, *Saccharomyces cerevisiae*, has recently become an attractive option. In this study, to realize rapid production of 2,3-BDO, a flocculent, 2,3-BDO-producing *S. cerevisiae* strain YPH499/dPdAdG/BDN6-10/FLO1 was constructed from a previously developed 2,3-BDO-producing strain. Continuous 2,3-BDO fermentation was carried out by the flocculent strain in an airlift bioreactor. The strain consumed more than 90 g/L of glucose, which corresponded to 90% of the input, and stably produced more than 30 g/L of 2,3-BDO over 380 h. The maximum 2,3-BDO productivity was 7.64 g/L/h at a dilution rate of 0.200/h, which was higher than the values achieved by continuous fermentation using pathogenic bacteria in the previous reports. These results demonstrate that continuous 2,3-BDO fermentation with flocculent 2,3-BDO-producing *S. cerevisiae* is a promising strategy for practical 2,3-BDO production.

Keywords 2,3-Butanediol · Continuous fermentation · Flocculation · Metabolic engineering · *Saccharomyces cerevisiae*

Introduction

Because of the environmental problems associated with the combustion of fossil fuels, the use of renewable feedstocks, such as cellulose and hemicellulose, and their constituent sugars for the production of bio-based chemicals, such as those that can be used as fuels, by engineering metabolic pathways in various microorganisms has recently gained prominence [8]. Among the host microorganisms used for metabolic engineering, the yeast *Saccharomyces cerevisiae* is particularly attractive due to its safety and ease of use. *S. cerevisiae* is a non-pathogenic organism, has been classified as "generally recognized as safe," and has long been used to produce organic compounds like ethanol [15]. In addition, the fermentation and processing technologies for large-scale production for *S. cerevisiae* are well established.

2,3-Butanediol (2,3-BDO) is one of the most useful metabolites produced by microorganisms, as this compound and its derivatives can be used as fuels, resins, solvents, food additives, cosmetics, drugs, and fumigants [1]. The most efficient 2,3-BDO-producing bacteria are *Klebsiella pneumoniae* and *Klebsiella oxytoca* [1]. However, because these bacteria are pathogenic, large-scale production is limited because of strict safety regulations [1, 4]. *S. cerevisiae* is a microorganism that shows promise for the production of 2,3-BDO, and there have been several reports on the production of 2,3-BDO by metabolically engineered *S. cerevisiae* strains harboring the bacterial 2,3-BDO biosynthetic pathway [9–11, 13]. We previously constructed an efficient 2,3-BDO-producing strain, YPH499/dPdAdG/BD6-10, which showed the highest 2,3-BDO production rate and yield in fed-batch fermentation reported thus far (Fig. 1) [26]. The strain lacked pyruvate decarboxylase *PDC1* and alcohol dehydrogenase *ADH1* to reduce byproduct ethanol production. Besides, the strain expressed α-acetolactate synthase and α-acetolactate decarboxylase from *Bacillus subtilis* and butanediol dehydrogenase from *Bacillus amyloliquefaciens* for 2,3-BDO biosynthesis. The strain also expressed NADH oxidase from *L. lactis* to address the redox imbalance issue. These four genes were expressed in an optimum ratio using a cocktail δ-integration strategy [25].

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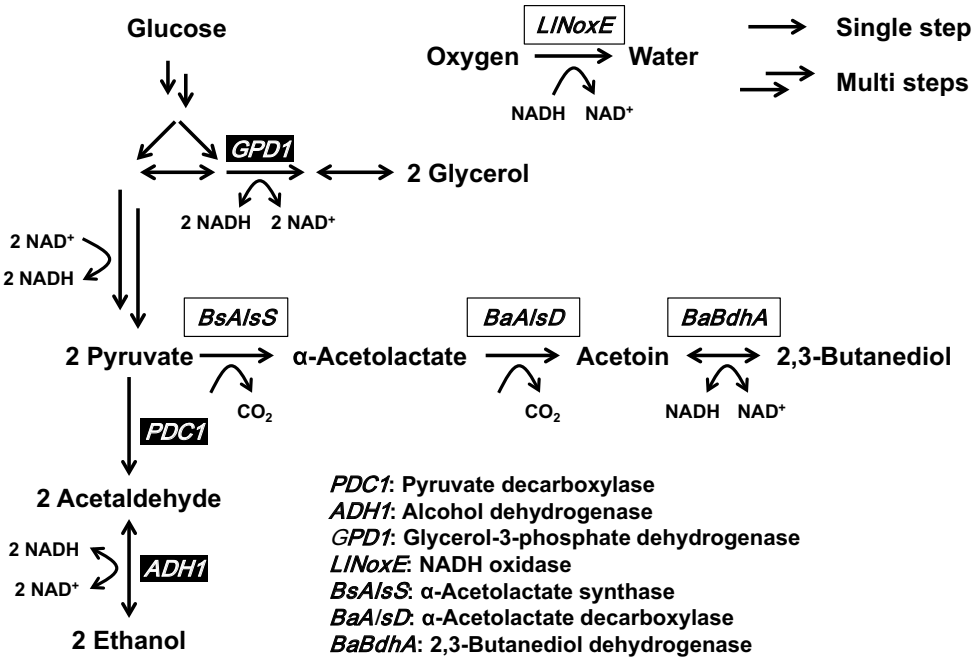

Fig. 1 Metabolic pathway for 2,3-butanediol production in YPH499/dPdAdG/BD6-10. Directional arrows and bidirectional arrows represent irreversible and reversible reactions, respectively. Genes indicated in the white boxes were overexpressed, and genes in the black boxes were deleted in this study

Although batch or fed-batch fermentation processes are widely used for the production of chemicals by various microorganisms, continuous fermentation is a good choice for practical production of bulk chemicals, such as 2,3-BDO, on a large scale, due to its high productivity and low labor and maintenance costs [24]. The high productivity in continuous fermentation is usually obtained by increased cell concentrations in the bioreactor, and immobilizing cells can provide high cell densities in the bioreactor. Among various immobilization techniques such as attachment to a surface and entrapment within a porous matrix, self-aggregation, which is known as flocculation, is very attractive due to its simplicity and low cost [20]. Various combinations of continuous bioreactor designs and yeast cell immobilization techniques have been studied [20]. Among these combinations, an airlift bioreactor combined with yeast flocculation is a promising process for bulk chemical production [20]. An airlift reactor gives vigorous circulation by a gas injection without mechanical agitation due to the internal draft tube, which creates a 'rising' zone in the center of the reactor and a 'down-coming' zone on the outside [20]. Thus, an airlift bioreactor realizes good agitation and low shear stress [6, 20]; these features are suitable for economical chemical production by flocculent yeast.

The purpose of this study was to realize rapid production of 2,3-BDO by continuous fermentation. We imparted flocculation ability to a previously developed 2,3-BDO-producing *S. cerevisiae* strain YPH499/dPdAdG/BDN6-10. Then, continuous 2,3-BDO fermentation was carried out by flocculent and non-flocculent 2,3-BDO-producing *S. cerevisiae* strains in an airlift bioreactor.

Materials and methods

Strains and media

Escherichia coli strain HST08 (TaKaRa Bio, Otsu, Japan) was used as a host for recombinant DNA manipulations. Recombinant *E. coli* cells were cultivated in Luria–Bertani (LB) medium (10 g/L tryptone [Nacalai Tesque, Kyoto, Japan], 5 g/L yeast extract [Formedium, Norfolk, UK], and 5 g/L NaCl), supplemented with 100 mg/L ampicillin sodium salt.

The previously developed 2,3-BDO-producing non-flocculent *S. cerevisiae* strain YPH499/dPdAdG/BDN6-10 [26] and the 2,3-BDO-producing flocculent *S. cerevisiae* strain YPH499/dPdAdG/BDN6-10/FLO1 were used for continuous 2,3-BDO fermentation. *S. cerevisiae* cells were cultivated in yeast/peptone/dextrose (YPD) medium (10 g/L yeast extract, 20 g/L peptone [Formedium], and 20 g/L glucose), YPD100 medium (10 g/L yeast extract, 20 g/L peptone, 100 g/L glucose, and 100 mg/L ampicillin sodium salt), or synthetic complete drop-out (SC) medium (6.7 g/L yeast nitrogen base without amino acids [Formedium], 20 g/L glucose, and 2 g/L SC multiple drop-outs [DSCK1028, Formedium]).

Yeast cultivation

Test tube cultivation was performed in 5 mL of YPD medium in a $\varphi 16 \times 165$ mm test tube on a reciprocal

shaker set at 30 °C and 150 rpm. Flask cultivation was performed in 150 mL of YPD medium in a 500-mL baffled flask on a rotary shaker set to 30 °C and 150 rpm. Cultivation was initiated by inoculating 1 mL of culture grown in a test tube for 72 h at 30 °C and 150 rpm in YPD medium.

Continuous airlift bioreactor cultivation was performed in 600 mL of YPD100 medium in a 1000-mL airlift bioreactor at 30 °C (Fig. 2a). Cultivation was initiated by inoculating the medium at an initial OD_{600} of 5.0 with a preculture grown in a flask, and water-saturated air was constantly supplied to the reactor at 2.0 vvm. After depleting the initial glucose, fresh YPD100 medium was fed at a predetermined dilution rate. An appropriate amount of antifoaming agent (Wako Pure Chemical, Osaka, Japan) was added as needed.

Construction and evaluation of the flocculent strain

All primers used in this study are summarized in Supplementary Table 1. The DNA fragment encoding the *PGK1* promoter from *S. cerevisiae* was amplified by PCR using *S. cerevisiae* YPH499 genomic DNA as the template with primers pPGK1F and pPGK1R. In addition, plasmid pRS405 (Stratagene, CA, USA), which contains the *LEU2* gene as a selective marker, was linearized by PCR using primers 405F and 405R. Then, the PCR products were conjugated using NEBuilder HiFi DNA Assembly master mix (New England BioLabs, MA, USA), and the resultant plasmid was named pRS405PG. Next, the DNA fragment used for replacement of the native *FLO1* promoter with the *PGK1* promoter was PCR amplified using plasmid pRS405PG as the template with primers FLO1-5dashF and FLO1-3dashR. Finally, the DNA fragment for replacement of the native

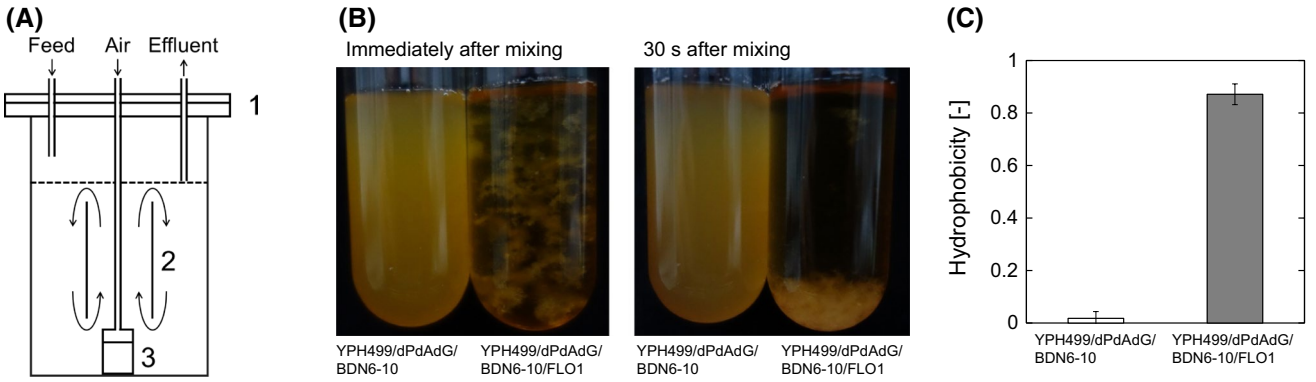

Fig. 2 **a** Schematic diagram of an airlift bioreactor. *1* Glass vessel (volume: 1 L, internal diameter: 85 mm); *2* draft tube (length: 70 mm, internal diameter: 55 mm); and *3* bubbling filter (pore size: 15 µm, material: stainless steel). The oxygen transfer coefficient kLa was calculated as 31.4/h; **b** flocculent behavior of engineered *Saccharomyces cerevisiae* cells immediately after mixing and 30 s after mixing. These strains were cultivated in YPD medium for 72 h at 30 °C and 150 rpm; **c** hydrophobicity of the engineered *Saccharomyces cerevisiae* strains. These strains were cultivated in YPD medium for 72 h at 30 °C and 150 rpm and subjected to the evaluation. Data are presented as the average of three independent experiments. Error bars are the standard deviations

Table 1 Glucose and metabolite concentrations and 2,3-BDO productivity in continuous fermentation

Saccharomyces cerevisiae strain	Phase	Dilution rate (per h)	Concentration in effluent (g/L)						2,3-BDO productivity (g/L/h)
			Glucose	2,3-BDO	Glycerol	Ethanol	Acetoin	Biomass	
YPH499/dPdAdG/BDN6-10	2	0.065	3.08 ± 5.45	33.50 ± 5.54	11.88 ± 8.70	0.00 ± 0.00	5.67 ± 9.24	4.05 ± 3.44	2.18 ± 0.24
	3	0.080	6.50 ± 11.26	34.53 ± 5.93	4.65 ± 5.34	0.00 ± 0.00	4.57 ± 4.39	9.49 ± 2.55	2.76 ± 0.47
	4	0.120	0.00 ± 0.00	38.14 ± 2.94	11.34 ± 5.14	0.00 ± 0.00	1.78 ± 1.81	7.14 ± 1.58	4.58 ± 0.35
	5	0.160	0.00 ± 0.00	36.26 ± 4.08	13.30 ± 3.11	0.00 ± 0.00	0.00 ± 0.00	9.17 ± 1.18	5.80 ± 0.65
	6	0.200	2.28 ± 3.94	33.64 ± 3.67	21.74 ± 5.13	0.00 ± 0.00	0.63 ± 1.10	6.41 ± 1.44	6.73 ± 0.73
YPH499/dPdAdG/BDN6-10/FLO1	2	0.065	5.87 ± 1.85	33.00 ± 2.62	24.62 ± 4.03	1.13 ± 1.16	0.14 ± 0.16	0.14 ± 0.06	2.15 ± 0.17
	3	0.080	4.32 ± 2.11	37.17 ± 0.93	28.21 ± 5.28	0.00 ± 0.00	0.06 ± 0.11	0.10 ± 0.05	3.00 ± 0.07
	4	0.120	7.37 ± 2.26	36.38 ± 0.52	35.04 ± 1.83	0.00 ± 0.00	0.09 ± 0.16	0.05 ± 0.03	4.37 ± 0.06
	5	0.160	4.32 ± 2.55	36.76 ± 1.79	41.42 ± 3.09	0.00 ± 0.00	0.31 ± 0.38	0.14 ± 0.12	5.88 ± 0.29
	6	0.200	6.51 ± 2.00	38.19 ± 1.76	42.17 ± 2.08	0.00 ± 0.00	0.26 ± 0.46	0.36 ± 0.28	7.64 ± 0.35

FLO1 promoter was transformed into *S. cerevisiae* YPH499/dPdAdG/BDN6-10 according to the previously described lithium acetate method [5]. Transformants were separated on SC medium containing 20-g/L agar and the appropriate amino acids and nucleic acids. The resultant strain was named YPH499/dPdAdG/BDN6-10/FLO1.

The hydrophobicity of yeast cells was evaluated as described previously [19], with some modifications. *S. cerevisiae* YPH499/dPdAdG/BDN6-10 and YPH499/dPdAdG/BDN6-10/FLO1 were cultivated in YPD medium for 72 h, and then harvested and washed twice with 50 mM EDTA/0.9% (w/v) NaCl (pH 8.5). The washed cells were resuspended in 50 mM EDTA/0.9% (w/v) NaCl (pH 8.5) at an OD_{600} of 1.0. Four hundred microliters of octane was added to 2 mL of cell suspension and vortexed at maximum speed for 60 s. After phase separation (10 min), the OD_{600} of the water layer was measured using an UVmini-1240 spectrophotometer (Shimadzu, Kyoto, Japan). The relative difference between the OD_{600} of the water layer before and after vortexing was considered as the hydrophobicity.

Analysis of cell growth and metabolites

The glucose, 2,3-BDO, glycerol, ethanol, acetoin, and biomass concentrations of culture broth were measured as described previously [26]. The carbon weight of the biomass was calculated using the conversion coefficient of 0.47 g of carbon/g of biomass [23]. The carbon yield of the biomass and metabolites was calculated using Eq. (1):

$$\text{Carbon yield } [\%] = \frac{\text{Carbon weight of biomass or metabolites produced [g]}}{\text{Carbon weight of glucose consumed [g]}} \times 100. \quad (1)$$

Results and discussion

Imparting flocculation ability to the 2,3-BDO-producing yeast strain

To impart flocculation ability to the 2,3-BDO-producing yeast strain, *FLO1*, which confers flocculation to yeast [21], was overexpressed in the 2,3-BDO-producing strain YPH499/dPdAdG/BDN6-10. As shown in Fig. 2b, the *FLO1*-overexpressing/2,3-BDO-producing strain YPH499/dPdAdG/BDN6-10/FLO1 showed clear flocculating behavior immediately after mixing when compared with the non-flocculent strain YPH499/dPdAdG/BDN6-10. Thirty seconds after mixing, almost all the YPH499/dPdAdG/BDN6-10/FLO1 cells precipitated, while the YPH499/dPdAdG/BDN6-10 cells barely precipitated (Fig. 2b). In addition, the YPH499/dPdAdG/BDN6-10/FLO1 cells showed significantly higher hydrophobicity than the YPH499/dPdAdG/BDN6-10 cells (Fig. 2c).

Flocculation ability was easily conferred by replacing the *FLO1* promoter with the *PGK1* promoter in YPH499/dPdAdG/BDN6-10 (Fig. 2b). Although commonly used laboratory strains such as S288C contain the five *FLO* genes *FLO1*, *FLO5*, *FLO9*, *FLO10*, and *FLO11* [3], these strains do not exhibit a flocculent phenotype due to a non-sense mutation in the *FLO* gene transcriptional regulator *FLO8* [19]. The parent strain used in this study (YPH499) is congenic with S288C [17]; thus, flocculation ability should be easily imparted by replacement of the *FLO1* promoter due to the presence of a functional *FLO1* gene.

YPH499/dPdAdG/BDN6-10/FLO1 cells showed significantly higher hydrophobicity than non-flocculent cells (Fig. 2c). The Flo1 protein has a hydrophobic C-terminal domain, and the hydrophobic interaction between Flo1 proteins is responsible for flocculation [2, 19]. Thus, the flocculent YPH499/dPdAdG/BDN6-10/FLO1 strain showed higher hydrophobicity than the non-flocculent YPH499/dPdAdG/BDN6-10 strain. Higher hydrophobicity in flocculent strains has been reported in some previous reports [19, 21].

Continuous 2,3-BDO fermentation in an airlift bioreactor

To evaluate the 2,3-BDO productivity of the non-flocculent and flocculent strains YPH499/dPdAdG/BDN6-10 and YPH499/dPdAdG/BDN6-10/FLO1, respectively, these strains were cultivated in a continuous airlift bioreactor, and the cell and compound concentrations in the culture effluent were evaluated. In phase 1, when the initial glucose input was exhausted, we started feeding fresh YPD100 medium (Fig. 3A). In phase 2, the dilution rate was set to 0.065/h for both strains. Thereafter, the dilution rate was incrementally increased from 0.080 to 0.200/h (in phases 3–6). The maximum dilution rate of 0.200/h was determined from the specific growth rate of YPH499/dPdAdG/BDN6-10 to prevent the washout [26]. After phase 2, the concentrations of 2,3-BDO and glucose appeared to be stable; thus, the values measured after phase 2 were used to evaluate 2,3-BDO productivity.

As shown in Fig. 3a and Table 1, both YPH499/dPdAdG/BDN6-10 and YPH499/dPdAdG/BDN6-10/FLO1 consumed more than 90 g/L of glucose, which corresponded to 90% of the input, and stably produced more than 30 g/L of 2,3-BDO through phases 2–6, which corresponding to more than 380 h. The maximum 2,3-BDO productivity in YPH499/dPdAdG/BDN6-10 and YPH499/

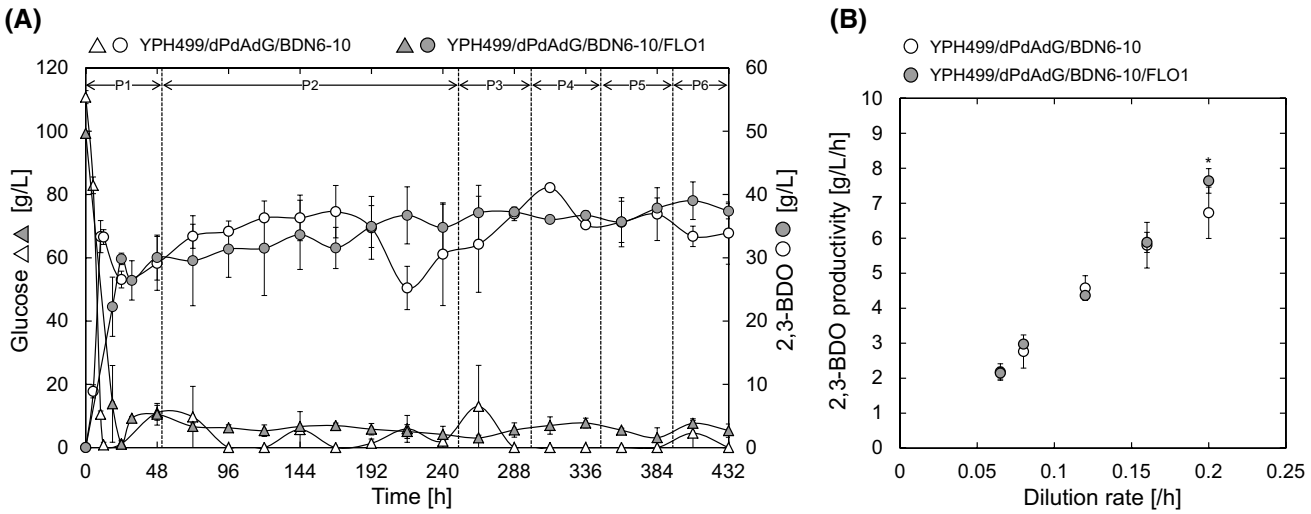

Fig. 3 **a** Time course of the changes in the glucose and 2,3-BDO concentrations during continuous fermentation by *Saccharomyces cerevisiae*. Data are presented as the average of two independent experiments. Error bars are the standard deviations. P1–6 are the phase numbers; **b** Correlation between the dilution rate and 2,3-BDO productivity in continuous fermentation. Data are presented as the average of two independent experiments. Error bars are the standard deviations. Significant differences were calculated using Student's *t* test (*$0.05 < p < 0.10$)

dPdAdG/BDN6-10/FLO1 was 6.73 and 7.64 g/L/h, respectively, at a dilution rate of 0.200/h.

As shown in Table 2, yields of the byproduct ethanol in both strains were significantly low during phases 2–6, whereas yields of the byproduct glycerol were relatively high, especially in YPH499/dPdAdG/BDN6-10/FLO1, and the yield increased with the dilution rate. The yields of the byproduct acetoin were also relatively high in YPH499/dPdAdG/BDN6-10. The biomass yield in YPH499/dPdAdG/BDN6-10 was significantly higher than that in YPH499/dPdAdG/BDN6-10/FLO1. The major byproducts in YPH499/dPdAdG/BDN6-10 were glycerol and biomass, and the major byproduct in YPH499/dPdAdG/BDN6-10/FLO1 was glycerol.

Although both the non-flocculent and flocculent strains produced glycerol as a major byproduct, the flocculent strain produced larger amount of glycerol than the non-flocculent strain. Both the non-flocculent and flocculent strains lacked major glycerol 3-phosphate dehydrogenase *GPD1* to reduce glycerol formation (Fig. 1) [26]. However, an isozyme *GPD2* exists in *S. cerevisiae*, and it is responsible for redox regulation [10]. High 2,3-BDO production causes a redox imbalance due to the production of an extra molecule of NADH in the chemical reaction in which one molecule of glucose is converted into one molecule of 2,3-BDO (Fig. 1) [10]. The formation of glycerol is one of the main redox balancing strategies, which regenerates of NAD^+, especially in *ADH1*/*PDC1*-deleted *S. cerevisiae* [14, 26]. Although YPH499/dPdAdG/BDN6-10 expressed NADH oxidase in

Table 2 Carbon yields of metabolites in continuous fermentation

Saccharomyces cerevisiae strain	Phase	Dilution rate (per h)	Carbon yield in effluent (%)				
			2,3-BDO	Glycerol	Ethanol	Acetoin	Biomass
YPH499/dPdAdG/BDN6-10	2	0.065	46.1 ± 7.3	16.3 ± 8.8	0.0 ± 0.0	7.8 ± 1.3	5.6 ± 4.1
	3	0.080	49.2 ± 3.3	6.6 ± 5.4	0.0 ± 0.0	6.5 ± 0.6	13.5 ± 2.1
	4	0.120	50.8 ± 3.9	15.1 ± 5.0	0.0 ± 0.0	2.4 ± 0.2	9.5 ± 1.9
	5	0.160	48.3 ± 5.4	17.7 ± 3.0	0.0 ± 0.0	0.0 ± 0.0	12.2 ± 1.4
	6	0.200	45.9 ± 5.7	29.6 ± 4.6	0.0 ± 0.0	0.9 ± 0.2	8.7 ± 1.6
YPH499/dPdAdG/BDN6-10/FLO1	2	0.065	46.7 ± 3.4	34.9 ± 4.2	1.6 ± 1.6	0.2 ± 0.0	0.2 ± 0.1
	3	0.080	51.8 ± 2.3	39.3 ± 6.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
	4	0.120	52.3 ± 1.5	50.4 ± 2.7	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	5	0.160	51.2 ± 3.1	57.7 ± 2.1	0.0 ± 0.0	0.4 ± 0.1	0.2 ± 0.1
	6	0.200	54.4 ± 3.5	60.1 ± 1.8	0.0 ± 0.0	0.4 ± 0.1	0.5 ± 0.3

Table 3 Comparison of microbial 2,3-BDO production by continuous fermentation

Strain	2,3-BDO (g/L)	2,3-BDO productivity (g/L/h)	References
Pathogenic bacteria			
Enterobacter aerogenes DSM 30053	43	5.6	[27]
Klebsiella pneumoniae NCIB 8017	Not determined	2.3	[12]
K. pneumoniae NRRL B199	35	4.3	[16]
Klebsiella sp. Zmd30	35	2.8	[22]
Recombinant *Saccharomyces cerevisiae* strains			
YPH499/dPdAdG/BD6-10	34	6.7	This study
YPH499/dPdAdG/BD6-10/FLO1	38	7.7	This study

an optimum ratio to resolve the redox imbalance, its ratio is affected by the concentration of dissolved oxygen [26]. Importantly, the flocculent strain YPH499/dPdAdG/BDN6-10/FLO1 exists in an anaerobic microenvironment inside a floc [18], which could lower the efficiency of NADH conversion to NAD^+ by NADH oxidase, resulting in an accumulation of a large amount of glycerol. Re-optimization of the 2,3-BDO production and NAD^+ regeneration pathways including glycerol formation in the engineered strains evaluated in this study should increase the yield of 2,3-BDO.

Total carbon yields through phases 4–6 in the flocculent strain YPH499/dPdAdG/BDN6-10/FLO1 slightly exceeded 100% (Table 2). This would be because microscopic heterogeneity of culture medium. The flocculent strain YPH499/dPdAdG/BDN6-10/FLO1 formed larger floc in later fermentation phase due to higher biomass concentration (data not shown). This large floc may prevent a smooth circulation in the airlift bioreactor. Optimization of the design of an airlift bioreactor should be required for more efficient continuous fermentation of 2,3-BDO by the flocculent strain YPH499/dPdAdG/BDN6-10/FLO1.

In the flocculent strain YPH499/dPdAdG/BDN6-10/FLO1, the concentration of biomass in the effluent was significantly lower than that in the non-flocculent strain; thus, these cells were efficiently retained inside the reactor due to flocculation. In fact, the biomass concentration inside the reactor of the flocculent strain YPH499/dPdAdG/BDN6-10/FLO1 (65.1 ± 0.0 g-dry cell/L) after 432 h fermentation was 3.0-fold higher than that of YPH499/dPdAdG/BDN6-10 (22.0 ± 0.7 g-dry cell/L). As shown in Fig. 3b, 2,3-BDO productivity by both the non-flocculent and flocculent strains increased proportionally with the dilution rate when the dilution rate was between 0.065 and 0.160/h. However, when the dilution rate was 0.200/h, the flocculent strain showed a proportional increase in 2,3-BDO productivity, whereas the non-flocculent strain did not. Thus, using the flocculent strain, more rapid 2,3-BDO production may be realized at an increased dilution rate. Scaling up of a reactor for continuous 2,3-BDO fermentation using the flocculent strain should be consider in future studies.

Although continuous fermentation is a good choice for 2,3-BDO production due to its high productivity, fed-batch fermentation processes are widely used due to their simplicity [7]. Some researchers have reported continuous fermentation of 2,3-BDO using pathogenic bacteria (Table 3). Zeng et al. reported the continuous fermentation of 2,3-BDO using pathogenic bacteria *E. aerogenes* in a continuous stirred tank reactor [27]. They achieved relatively higher 2,3-BDO productivity by optimizing oxygen uptake rate. Besides, Ramachandran and Goma reported the continuous fermentation of 2,3-BDO using pathogenic bacteria *K. pneumoniae* in a continuous stirred tank reactor [16]. To our knowledge, we are the first to report continuous 2,3-BDO fermentation using non-pathogenic engineered *S. cerevisiae* strains, and these strains showed higher 2,3-BDO productivity, with a comparable 2,3-BDO titer, than these pathogenic bacteria. Although the 2,3-BDO titer and yield should be improved to allow comparison of the yield in fed-batch fermentation to that of various microorganisms [7], continuous 2,3-BDO fermentation using engineered *S. cerevisiae* is a promising strategy for 2,3-BDO production.

Conclusion

Rapid and stable continuous 2,3-BDO fermentation was successfully achieved using a previously developed non-pathogenic engineered *S. cerevisiae* strain; imparting flocculating ability to the 2,3-BDO-producing strain generated a strain capable of producing 2,3-BDO at a higher rate. Improvement of the 2,3-BDO titer and yield using this flocculent yeast and scaling up of the reactor are needed to confirm the feasibility for practical, continuous 2,3-BDO fermentation using an engineered yeast.

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