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Effects of culture condition and nutrition on the co-production of microbial oil and exopolysaccharide by *Sporidiobolus pararoseus* JD-2

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Microbial oil has been gaining considerable attention from researchers recently as renewable and ecofriendly oil and its potential as feedstock for food industry and biodiesel industry. In this context, we have earlier demonstrated production of microbial oil and exopolysaccharide (EPS) from the yeast *Sporidiobolus pararoseus* JD-2. In this study, we explored increasing its production by optimizing the culture condition and nutrition. As expected, culture temperature and dissolved oxygen (DO) are the contributing factors for co-producing microbial oil and EPS, in which 28°C and lower quantum (i.e., 30 mL/500 mL) show the best conditions in shake-flasks fermentation. By contrast, the initial pH from 4 to 8 has no obvious effect on producing microbial oil and EPS. In addition, the culture nutrition (i.e., carbon/nitrogen source) were also discussed, and indicating that 20 g/L of corn steep liquor and 60 g/L of glucose are beneficial to produce microbial oil and EPS (i.e., 34.1±1.2 g/L and 11.5±0.2 g/L, respectively). Meanwhile, the residue glucose should be maintained at 20 g/L, in which the highest production of microbial oil and EPS was obtained (i.e., 34.6±1.7 g/L and 11.7±0.8 g/L, respectively). The biomass, microbial oil and EPS were further increased during optimizing the DO level, which reached to 67.8±2.1 g/L, 34.7±0.6 g/L and 11.8±0.5 g/L during maintaining DO level at 20-30%, respectively. The results suggest that appropriate culture condition and nutrition considerably improve the fermentation performance of *S. pararoseus* JD-2 and significantly increase co-production of microbial oil and EPS (by 11.2 and 8.3%, respectively) compared to the un-optimized fermentation.

Keywords: Culture condition, Culture nutrition, Dissolve oxygen, Yeast

An oleaginous yeast (i.e., *Sporidiobolus pararoseus* JD-2, CCTCC M2010326) was isolated from bean-based sauce in 2010 by Han *et al*.[1], which co-produced microbial oil, exopolysaccharide (EPS) and carotenoid[2,3]. Microbial oil, as renewable and eco-friendly oil, can be used as potential feedstock for food industry and biodiesel industry because the composition of fatty acids in microbial oil is close relative to vegetable oil[4,5]. EPS, as high value-added product, can be applied to food and medicine industry[6]. However, high production cost limits the broad application of microbial oil and EPS because of low yield and poor stability of producing strains. Many attempts have been done by researchers to overcome such limitations, particularly (i) breeding the high yield strains by genetic engineering methods and/or traditional breeding technologies[7]; (ii) using cheaper raw materials as feedstock[8,9]; (iii) optimizing the culture methods, e.g., mixed cultivation[10]; and (iv) co-producing microbial oil and EPS[11]. In our previous studies, Han *et al*.[3] and Wang *et al*.[12] tried to increase the production of microbial oil and EPS in *Sporidiobolus pararoseus* JD-2 by optimizing the composition of nitrogen source and limiting the ammonia-nitrogen supply. Recently, Guo *et al*.[9] tried to further increase the production of microbial oil and EPS by controlling the foams formation in broth. Although these methods make some results in the practical application, inappropriate culture condition and nutrition also led to increased production cost and decreased production of microbial oil and EPS. Controlling the culture condition and nutrition is the key for optimal yield.

Culture condition (viz. culture temperature, pH, dissoluble oxygen and inoculum amount) and culture nutrition (carbon source and nitrogen source) are

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Abbreviations: CSL, Corn steep liquor; CSP, Corn steep power; DO, Dissolved oxygen; EPS, Exopolysaccharide

closely related to cell growth and metabolic activity of bacteria, and thus affect the yield of products by microbiological fermentation. In order to ensure the stability and repeatability of fermentation performance, culture temperature, pH and dissoluble oxygen have been monitored. Some scientists contend that increasing agitation speed and ventilatory capacity to improve dissolved oxygen (DO) level is conducive to EPS production[13]. On the contrary, some kinds of EPS (e.g., alginate and xanthan gum) were produced at low DO level because the agitation speed will disrupt the cell structure or change the physical and chemical properties of EPS[14]. It should be noted that the low DO level will inhibit the cell growth. However, how to control the DO level in the fermentation progress of co-producing microbial oil and EPS have not been reported in literatures as far as we know. In addition, culture temperature and pH affect the compositions and viscosity of medium and thus affecting the metabolic activity of bacteria and the stability and formation of foam[15].

Besides the culture condition, the appropriate culture nutrition is also critical for co-producing microbial oil and EPS. Cultivation of producing strain in the broth with limited nutrition (ammonia nitrogen-limited, phosphorus-limited and sulphur-limited) has been proved to be good for producing microbial oil and EPS[12]. In 2017, Shen *et al*.[16] found that the highest microbial oil production (10 g/L) and oil content (55%) were obtained when the C/N ratio was 72, whereas the microbial oil production and oil content were decreased by 50-60% when the C/N ratio was 24. This is because that the surplus carbon source will be re-directed into carbon metabolic pathway, and thus to increase the triglyceride accumulation rather than to use for cell growth. However, the high concentration of carbon source will be detrimental to the target products production because the high osmotic pressure limits the cell growth[17]. In addition, excessive addition of carbon source will lead to increase production cost and to waste raw materials. Although the above mentioned strategies on controlling culture condition and nutrition have achieved positive results, the best fermentation performance was regulated by complex interactions among the above mentioned factors. Therefore, extensive research is still needed to optimize the culture condition and nutrition.

In 2022, we achieved increased production of microbial oil and EPS by controlling the foams formation in broth[9]. On the basis of aforesaid work, here, in the present study, we tried to further increase the production of microbial oil and EPS from the yeast strain *Sporidiobolus pararoseus* JD-2 by optimizing the culture condition and nutrition in broth. To do this, the effect of culture temperature, pH and inoculum amount was firstly discussed. Subsequently, carbon source and nitrogen source were optimized in fed-batch fermentation by *S. pararoseus* JD-2, including optimizing initial concentration and residual concentration. Lastly, we determined the best DO level as well.

Materials and Methods

Strain and the initial culture conditions

The microbial oil- and EPS- producing yeast strain *S. pararoseus* JD-2 was isolated from bean-based sauce in our laboratory[1], which was stored in China Center for Type Culture Collection (CCTCC, Accession Number: M2010326). *S. pararoseus* JD-2 was cultured in YPD plates (Yeast extract 10 g/L, Peptone 20 g/L, Dextrose 20 g/L) and at 28°C and pH 6.0 for 72 h. The fed-batch fermentation was performed in a 7-L jar fermenter (KF-7 L, Korea Fermenter Co., Inchon, Korea) with a foam backflow device. And the inoculum size was 10% (v/v) from a seed culture grown to $\Delta OD_{600} \approx 0.8$ (at a dilution of 25-fold). The seed medium consisted of (g/L): glucose 30, corn steep liquor (CSL, provided by Shandong Shouguang Juneng Golden Corn Co., Ltd.) 20, KH_2PO_4 1, $MgSO_4 \cdot 7H_2O$ 0.5. The initial culture medium used for fermentation contained (g/L): glucose 120, CSL 20, $(NH_4)_2SO_4$ 1.2, K_2HPO_4 1, Na_2SO_4 0.1. Unless otherwise stated, the temperature and dissolved oxygen (DO) level were set at 28°C and 20%, respectively. The 800 g/L sterile glucose solution was used to control the glucose concentration and the pH in medium were adjusted using 20% (m/v) NaOH.

Optimization of culture condition

The culture conditions, including temperature, pH and DO, were optimized in shake flasks and/or fermenter. For optimizing temperature, the test temperature (i.e., 25, 28, 30, 35 and 37°C) was controlled by heater equipped on incubator or fermenter. The pH was controlled at 4, 5, 6, 7 or 8 by feeding 36.5 g/L HCl or 20% (m/v) NaOH, respectively. For optimizing the DO level, the loaded liquid volume in 500 mL flask (i.e., 30, 50, 80, 100 and 150 mL/500 mL) was used to analyze the DO level of mediums in shake-flasks and the OD level of

mediums in fermenter was controlled by regulating aeration volume and agitation speed in 5-10%, 10-20, 20-30 and 30-40%, respectively.

Optimization of culture nutrition

The culture nutrition, including organic nitrogen source and carbon source, were optimized in shake-flasks and/or fermenter. Firstly, four kinds of organic nitrogen source (i.e., yeast extract, CSL, corn steep powder and peptone) were used to screen the best nitrogen source for co-producing microbial oil and EPS by *S. pararoseus* JD-2. After that, the concentration of the target nitrogen source was optimized to identify the best concentration of the target nitrogen source for co-producing microbial oil and EPS in Mode IV, in which a half of nitrogen source was added in the medium and the other half of nitrogen source was fed in medium at between 8 ~ 24 h[9]. Six gradients of the target nitrogen source (i.e., 10, 15, 20, 25. 30 and 40 g/L) were used to determine the best concentration of the nitrogen source for co-producing microbial oil and EPS.

For optimizing carbon source, the initial concentration of glucose was firstly discussed and four gradients of glucose (i.e., 30, 60, 90 and 120 g/L) were used to determine the best concentration of glucose for co-producing microbial oil and EPS. Subsequently, the residual glucose concentration was controlled by feeding 800 g/L sterile glucose solution to maintain the residual glucose concentration at 5, 10, 20 and 30 g/L, respectively.

Extraction of microbial oil and EPS

A sample was taken out from shake-flasks or fermenter every 4 h and then centrifuged at 10,000 r/min for 20 min. The cell pellets were used to extract microbial oil and the culture supernatants were used to extract EPS. The extraction process of microbial oil and EPS has been reported[3,12]. For extraction of microbial oil, the cell pellets were incubated in 18.25 g/L HCl at 60℃ and 10 min for disrupting cells and then 20 mL ethyl acetate were added and incubated for 30 min after centrifuging 10,000 × *g* for 10 min. After that, the leaching solution was evaporated in a rotary evaporator at 50℃. In addition, the culture supernatants was mixed with 95% ethanol at a 1:2 (v/v) ratio and sedimentated in nature for 2 h, and then the EPS was collected by centrifugation at 10,000 × *g* for 25 min and dried by freeze drier.

Analytical methods

Biomass was measured using a spectrophotometer at 600 nm after an appropriate dilution. Microbial oil and EPS were analyzed by weight after extraction, and the detail processes were referred to our previous description[3,12]. The oil content was defined as: oil content = (microbial oil / biomass) × 100%. The residual glucose concentration was determined using an SBA-50B biosensor analyzer (Shandong Academy of Sciences, Jinan, China), and the NH_4^+ concentration was determined by the indophenol method[18]. The analyses of biomass, microbial oil and EPS were done in triplicate_ENREF_23.

Statistical analysis

All of experiments in this study were independently performed at least three times, and data are represented as mean and standard deviation (±SD). Student's *t* test was used for comparing the statistical difference among the groups of experiment data_ENREF_44_ENREF_26.

Results and Discussion

Hunting for key culture conditions for co-producing microbial oil and EPS by *S. pararoseus* JD-2

Culture conditions, e.g., temperature, dissolved oxygen (DO) and pH, showed a multiple effect on cell growth and product biosynthesis for bacteria. For example, temperature and pH affect the enzymatic activity and membrane permeability, thereby can affect the catabolism of substrates in bacteria[19,20]. In addition, oxygen is also an important factor for cell growth and product biosynthesis in bacteria, especially for aerobic bacteria[21]. In order to discuss the key culture conditions for co-producing microbial oil and EPS by *S. pararoseus* JD-2, the temperature, loaded liquid volume and pH were investigated in shake-flasks fermentation. The test culture conditions showed the different effect on producing microbial oil and EPS by *S. pararoseus* JD-2 (Fig. 1). As can be seen from Fig. 1A, the temperature has obvious effect on producing microbial oil and EPS, and the highest cell growth and products yield (i.e., microbial oil and EPS) were found at 28℃. Based on the knowledge reported by Han *et al.*[1], *S. pararoseus* JD-2 grows best when temperature is 28℃. This could explain why 28℃ show the best temperature for cell growth and the production of microbial oil and EPS. Besides temperature, DO is also the key factor for co-producing microbial oil and EPS, and lower quantum (i.e., 30 mL/500 mL) show the best volume for cell growth and for producing microbial oil and EPS (Fig. 1B). Since *S. pararoseus* JD-2 is an aerobic bacteria[1], thus more oxygen was needed to maintain the cell growth with high rate. In addition, many researches indicated that the high DO is beneficial to

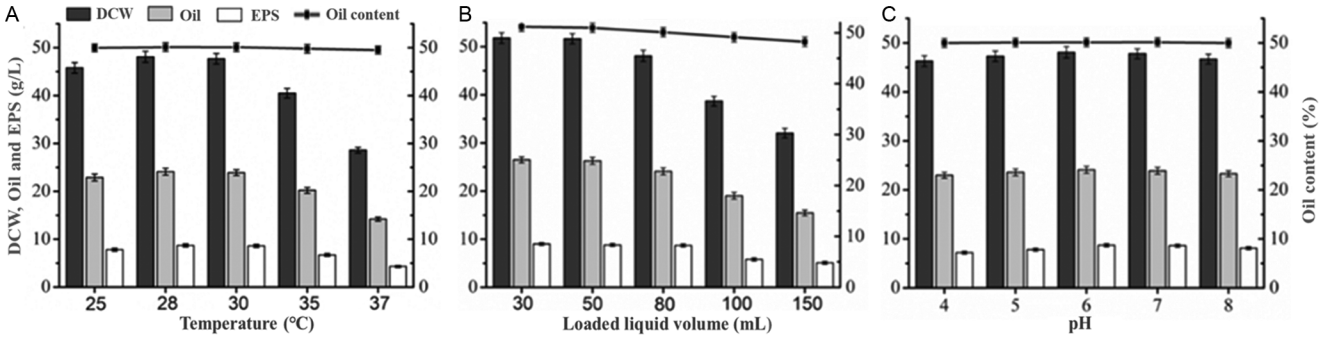

Fig. 1 — Optimization of culture conditions of *Sporidiobolus pararoseus* JD-2 in shake-flasks fermentation. Effect of (A) temperature; (B) loaded liquid volume; and (C) pH on *S. pararoseus* JD-2 fermentation. The data represent mean values and standard deviations obtained from three independent cultivations.

increase the production of microbial oil and/or EPS[9,13]. It should be noted that the initial pH in the test range has no obvious effect on cell growth and producing microbial oil and EPS (Fig. 1C). *Sporidiobolus* sp. (including *S. pararoseus*) has evolved some unique tolerant mechanism in response to salt stress or other extreme factors [22], and it can used non-carbohydrate substrates (e.g., citrate and acetate[23]). And our results indicated that the cell growth of *S. pararoseus* JD-2 would be obvious inhibited during pH ≤4.0 or pH ≥8.0 (data not shown). Therefore, we speculated that *S. pararoseus* JD-2 has a better adaptive ability to acidic. Given that the pH in broth is closely associated with protein solubility[24] and foams foaming[9], the best pH in broth was set at 6.0.

As mentioned above, culture conditions affect the cell growth and products yield. There results indicate that 28℃, lower quantum and pH 6.0 were the best culture conditions for co-producing microbial oil and EPS (Fig. 1). In order to master the changes of the fermentation parameters after optimization, some fermentation parameters (e.g., glucose, NH_4^+, DCW, microbial oil and EPS) were monitor in the whole fermentation process in the shake-flasks fermentation. As can be seen from Fig. 2, glucose and NH_4^+ were dramatically consumed while the cell growth was quickly increased at initial stage. In addition, microbial oil and EPS began to increase because NH_4^+ was completely consumed after cultivating for 12 h (Fig. 2). The similar results were also reported by Osman *et al.*[25] and Wang *et al.*[12], in which nitrogen-limited condition was considered the key factor to produce microbial oil and EPS. The maximal cell growth (51.8±1.6 g/L), microbial oil (26.5±0.6 g/L) and EPS (8.9±0.3 g/L) were achieved under optimal culture conditions, i.e., 28℃, pH 6.0 and 30 mL/500 mL flask.

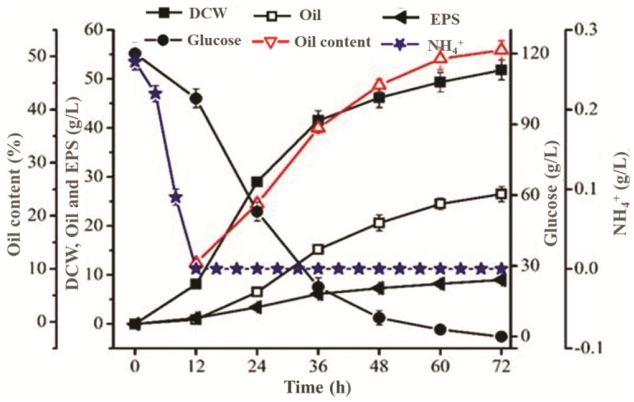

Fig. 2 — *Sporidiobolus pararoseus* JD-2 fermentation curve under optimized culture conditions.

Optimizing organic nitrogen source to enhance co-production of microbial oil and EPS by *S. pararoseus* JD-2

As noted earlier, microbial oil and EPS began to increase in the nitrogen-limited condition (Fig. 2)[12]. However, nitrogen source is necessary nutrition to support cell life. In order to obtain the best nitrogen source and its optimal concentration to balance the cell growth and the production of microbial oil and EPS by *S. pararoseus* JD-2, we investigated the effect of different organic nitrogen sources on cell growth and products yield. And on this basis we further optimized the concentration of the best organic nitrogen source. As expected, different organic nitrogen sources showed the different effect on cell growth and products yield (Fig. 3A). Among these test organic nitrogen sources, CSL showed the best capacity to maintain cell growth and products yield (Fig. 3A). Although the cell growth during using CSL as organic nitrogen source is lower than that of the control group (i.e., using 10 g/L yeast extract and 10 g/L tryptone), the oil content is obvious higher than that of the control group. The similar results were also reported in previous research, in which

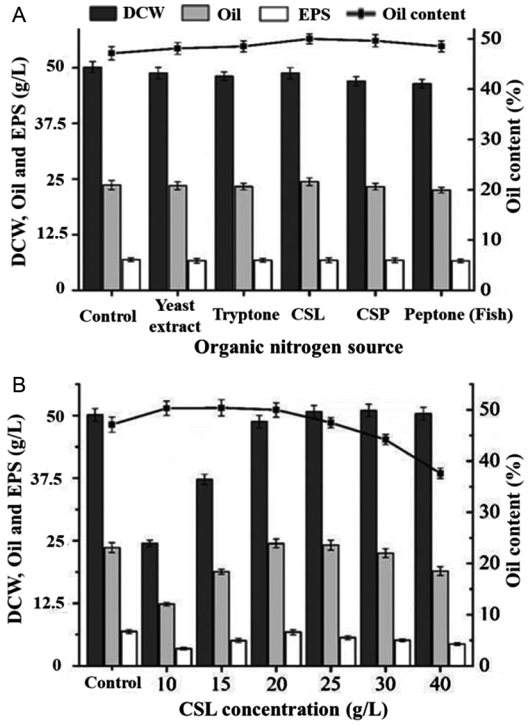

Fig. 3 — Effects of organic nitrogen sources on fermentation performance of *Sporidiobolus pararoseus* JD-2. Effects of (A) different organic nitrogen sources; and (B) different CSL concentration on *S. pararoseus* JD-2 fermentation. [CSP represents corn steep powder. The data represent mean values and standard deviations obtained from three independent cultivations]

yeast extract was found as the better organic nitrogen source for cell growth than CSL[3]. This is possible because of the different protein content in different organic nitrogen sources, however too much protein will inhibit the biosynthesis of microbial oil[12]. Based on the above mentioned results, CSL was used as the preferred organic nitrogen source during fermentation by *S. pararoseus* JD-2 in the next study.

Previous study reported by Han *et al.*[3] and our previous research[9] indicated that the concentration and feeding mode of CSL affect the production of microbial oil and EPS. Thus, the concentration of CSL was optimized in next study to identify the best initial CSL concentration for co-producing microbial oil and EPS in Mode IV[9]. As can be seen from Fig. 3B, the cell growth was increased along with the increase of CSL. However, the production and content of microbial oil and the production of EPS was oblivious decreased when CSL exceed 20 g/L. These results indicated that more carbon source was used to cell growth rather than to biosynthesize microbial oil and EPS because of the abundant nitrogen sources in medium[26]. Although the oil content was the highest at the 10 g/L of CSL, the production of microbial oil and EPS were the lowest. Compared with the other concentration of CSL, 20 g/L of CSL showed the best capacity for maintaining the higher cell growth, microbial oil yield and EPS yield (Fig. 3B). Therefore, 20 g/L of CSL is considered as appropriate concentration for co-producing microbial oil and EPS by *S. pararoseus* JD-2.

Table 1 — Effect of initial glucose concentration on fermentation performance of *S. pararoseus* JD-2

Initial conc. (g/L)	Biomass (g/L)	Microbial oil (g/L)	EPS (g/L)
120	62.3±1.8	31.2±0.8	10.9±0.4
90	66.8±1.7	33.3±1.1	11.5±0.5
60	67.4±1.6	34.1±1.2	11.5±0.2
30	66.2±1.4	33.2±1.2	11.3±0.4

Determining best initial glucose concentration for co-producing microbial oil and EPS by *S. pararoseus* JD-2

Glucose, as the important nutrition for cell's life activity, controlled the metabolic function in cells at the logarithmic growth phase and the product biosynthesis phase[27]. In the logarithmic phase of growth, extra glucose makes the high accumulation of organic acids and the big demand for oxygen, whereas excessively low glucose leads the increase of pH and abnormal cell growth because the nitrogen source is used as carbon source for cell[28]. Therefore, we firstly investigated the effects of the initial concentration of glucose on fermentation performance of *S. pararoseus* JD-2. Contrary to expectations, the highest concentration of glucose (i.e., 120 g/L) was bad news for cell growth, thus inhibiting the production of microbial oil and EPS (Table 1). It could be partly due to the high osmotic pressure resulted from the high glucose concentration[29]. The highest biomass (i.e., 67.4±1.6 g/L), microbial oil (i.e., 34.1±1.2 g/L) and EPS (i.e., 11.5±0.2 g/L) was fund during adding 60 g/L glucose in medium, which increased by 8.2, 9.3 and 5.5% as compared with adding 120 g/L glucose, respectively (Table 1). These results indicated that the low glucose concentration is beneficial to cell growth and to products biosynthesis because of the low osmotic pressure and the low viscosity in medium. It should be noted that too little glucose also can be a problem for cell growth and products biosynthesis. For example, the biomass, microbial oil and EPS were decreased during adding 30 g/L of glucose as compared with adding 60 g/L of glucose (Table 1). This is probably due to the shortage of carbon source for cell growth in the early fermentation stage. Based

on the above mentioned results, 60 g/L of glucose was used as the best initial glucose concentration during fermentation by *S. pararoseus* JD-2 in the next study.

Regulating residual glucose concentration to maintain the best C/N ratio in co-producing microbial oil and EPS from *S. pararoseus* JD-2

As mentioned above (Table 1), the glucose concentration in medium is important for cell growth and products biosynthesis. In addition, the high C/N ratio in products biosynthesis phase is beneficial to the production of microbial oil and EPS[3,12]. In order to balance the cell growth and products biosynthesis in fed-batch fermentation by *S. pararoseus* JD-2, we investigated the effect of residual glucose in broth on the production of microbial oil and EPS. As can be seen from Fig. 4A, the excessively low residual glucose (e.g., 5 g/L) inhibited the cell growth and thus decreasing the production of microbial oil and EPS. By contrast, the excessively high residual glucose (e.g., 30 g/L) was also bad for increasing microbial oil and EPS production although it was good for cell growth (Fig. 4A). The similar results were also found in previous research[30], in which *Rhodosporidium toruloides* CBS14 showed the highest production rate, yield and content of microbial oil in the culture with 65 g/L of initial glucose and feeding low glucose. We speculated that too little glucose is not adequate for cell growth but too much glucose was used to biosynthesize the by-products (e.g., acetate) rather than the target products (i.e., microbial oil and EPS)[31]. Within the range of test residual glucose concentration, the highest microbial oil and EPS were obtained when the residual glucose concentration was maintained at 20 g/L, in which microbial oil reached to 34.6±1.7 g/L and EPS reached to 11.7±0.8 g/L (Fig. 4A). These results indicated that the rational C/N ratio in fermentation broth is a key factor for increasing microbial oil and EPS production. In addition, maintaining the rational residual glucose in broth is also beneficial to control the production cost and to decrease the viscosity of broth.

Controlling dissolved oxygen level to further increase co-production of microbial oil and EPS from *S. pararoseus* JD-2

Previous research indicated that DO level affects accumulation of microbial oil in intracellular and the production of EPS[9,14]. In order to further increase the co-production of microbial oil and EPS by *S. pararoseus* JD-2, four DO levels were investigated during fed-batch fermentation at the nutrition of adding 20 g/L of CSL and 60 g/L of glucose as well as maintain 20 g/L of residual glucose in broth. As expected, the DO level is very important for cell growth and the production of microbial oil and EPS, in which the biomass and the production of microbial oil and EPS are proportionate to the DO level (Fig. 4B). These results are consistent with the results performed in shake-flasks fermentation (Fig. 1B) and with the previous results reported by Liu *et al.*[13]. This is because *S. pararoseus* JD-2 is an aerobic bacterium [1], and thus oxygen is one indispensable factor for cell

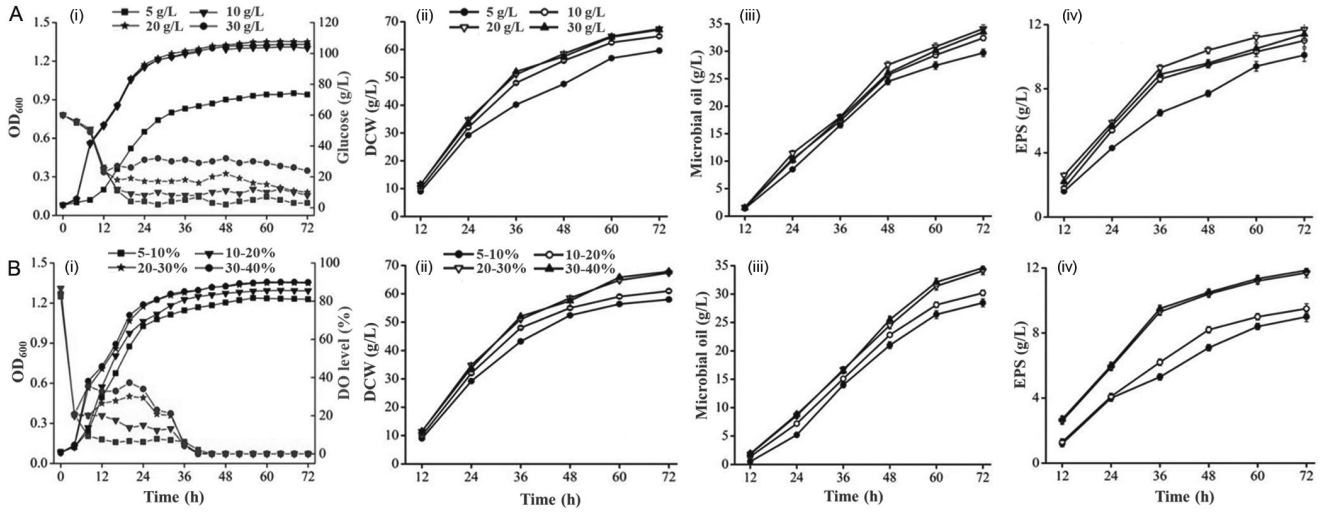

Fig. 4 — Effect of (A) residual glucose; and (B) dissolved oxygen (DO) level on the fermentation of *Sporidiobolus pararoseus* JD-2. (i) Effect of residual glucose\DO level on cell concentration (OD_{600}) of *S. pararoseus* JD-2, (ii) Effect of residual glucose\DO level on biomass (Dry cell weight, DCW) of *S. pararoseus* JD-2, (iii) Effect of residual glucose\DO level on microbial oil production by *S. pararoseus* JD-2, and (iv) Effect of f residual glucose\DO level on EPS production by *S. pararoseus* JD-2. [The data represent mean values and standard deviations obtained from three independent cultivations]

growth. In addition, the biosynthesis of microbial oil and EPS needs the participation of oxygen to supply energy[32]. The cell growth was obviously inhibited when the DO level is below 20% [Fig. 4B (i) & (ii)], thus reducing the production of microbial oil and EPS [Fig. 4B (iii) & (iv)]. As compared with setting DO level at 5-10%, the biomass and the production of microbial oil and EPS were increased by 16.5% (58.2±1.1 g/L *vs*. 67.8±1.5 g/L), 22.5% (28.5±0.8 g/L *vs*. 34.9±1.1 g/L) and 32.2% (9.0±0.5 g/L *vs*. 11.9±0.3 g/L) at setting DO level at 30-40%, respectively. It is worth noting that maintaining the high DO level led to a lot of bubbles forming (Data not shown). The similar results were also found in our previous results[9], in which air entrapment was considered positive factor to foam formation. In addition, although the highest biomass and production of microbial oil and EPS were found in setting DO level at 30-40%, the biomass and the production of microbial oil and EPS during DO level at 20-30% were not obvious lower than that during DO level at 30-40% (Fig. 4B). The biomass and the production of microbial oil and EPS during DO level at 20-30% were 67.8±2.1 g/L, 34.7±0.6 g/L and 11.8±0.5 g/L, respectively. Combined with the above reasons, therefore, the DO level was set at 20-30% for co-producing microbial oil and EPS in fed-batch fermentation.

Conclusion

The appropriate culture condition and nutrition are crucial for producing chemical by microbiological fermentation, especially for co-producing two or more chemicals. In this study, we pointed out that culture temperature and dissolved oxygen (DO) level are the key factors for co-producing microbial oil and EPS while the initial pH in the test range has no obvious effect on producing microbial oil and EPS. And we found that the rational initial glucose concentration and residual glucose concentration as well as the nitrogen source are crucial to increase the production of microbial oil and EPS. 20 g/L of corn steep liquor (CSL) showed the best nitrogen source for co-producing microbial oil and EPS. In addition, adding 60 g/L of glucose in medium and maintaining the residual glucose at 20 g/L in broth resulted in the increased biomass, microbial oil yield and EPS yield. DO level in fed-batch fermentation was once again proved to beneficiate for co-producing microbial oil and EPS, in which setting DO level at 20-30% resulted in the highest biomass and production of microbial oil and EPS. Under such optimized culture condition and nutrition, the yeast strain *Sporidiobolus pararoseus* JD-2 produced 34.7±0.6 g/L of microbial oil and 11.8±0.5 g/L of EPS, which was increased by 11.2 and 8.3% as compared with the original condition and nutrition (i.e., 31.2±0.8 g/L of microbial oil and 10.9±0.4 g/L of EPS), respectively. The results have once again proved that appropriate culture condition and nutrition considerably improve the fermentation performance of *S. pararoseus* JD-2.

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Conflicts of interest

Authors declare no competing interests.

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