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Research article

Strategy of oxygen transfer coefficient control on the L-erythrulose fermentation by newly isolated *Gluconobacter kondonii*

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ABSTRACT

Background: The effect of diverse oxygen transfer coefficient on the L-erythrulose production from meso-erythritol by a newly isolated strain, *Gluconobacter kondonii* CGMCC8391 was investigated. In order to elucidate the effects of volumetric mass transfer coefficient (k_La) on the fermentations, baffled and unbaffled flask cultures, and fed-batch cultures were developed in present work.

Results: With the increase of the k_La value in the fed-batch culture, L-erythrulose concentration, productivity and yield were significantly improved, while cell growth was not the best in the high k_La. Thus, a two-stage oxygen supply control strategy was proposed, aimed at achieving high concentration and high productivity of L-erythrulose. During the first 12 h, k_La was controlled at 40.28 h^{-1} to obtain high value for cell growth, subsequently k_La was controlled at 86.31 h^{-1} to allow for high L-erythrulose accumulation.

Conclusions: Under optimal conditions, the L-erythrulose concentration, productivity, yield and DCW reached 207.9 ± 7.78 g/L, 6.50 g/L/h, 0.94 g/g, 2.68 ± 0.17 g/L, respectively. At the end of fermentation, the L-erythrulose concentration and productivity were higher than those in the previous similar reports.

1. Introduction

L-Erythrulose is a known active compound for spontaneous tanning agents and is employed on its own or, for example, together with a further compound such as a reducing sugar having spontaneous tanning properties for artificial tanning of the skin [1]. In the cosmetics industry, L-erythrulose can act as a substitute for dihydroxypropanone (DHA) solving the problem most people allergic to DHA [2]. It has been widely used in the field of chemical industry and medical. With hydroxyl and carbonyl, L-erythrulose has more reactive chemical properties, and it can participate in chemical reactions, such as polymerization, condensation as medicine and pesticide intermediate synthesis, synthesis of heterocyclic compounds such as imidazole, furan, and substitution compound synthesis [3].

There have been some reports on the production of L-erythrulose from meso-erythritol. The biosynthesis of L-erythrulose is known, thus, for example, for the biotransformation of meso-erythritol to L-erythrulose by means of *Acetobacter suboxydans* (ATCC 621) in a medium comprising peptone and yeast or yeast extract and calcium carbonate was described by Whistler and Underkofler [4]. A disadvantage in this process is the low conversion yield of 45–50% L-erythrulose from the added meso-erythritol. Imfeld et al. [5] established a novel biotechnological process for the preparation of L-erythrulose from meso-erythritol using *Gluconobacter oxydans* in a 15 m^3 bioreactor, the concentration of L-erythrulose reached 69.4 g/L after 31 h. The resting cell transformation with 10% of the substrate concentration was used by Mizanur et al. [6] in 2001. After 48 h, the final conversion rate reached 98%, productivity of only 2.04 g/L/h. Moonmangmee et al. [7] reported that *Gluconobacter frateurii* CHM 43 was screened among thermotolerant *Gluconobacter* and mesophilic strains for L-erythrulose production from meso-erythritol when grown at 37°C in 2002. *Gluconobacter* strains produce L-erythrulose via incomplete oxidation of meso-erythritol with the activity of membrane-bound quinoprotein membrane-bound meso-erythritol dehydrogenase (QMEDH) (EC 1.1.1.162) [8]. The enzyme responsible for meso-erythritol oxidation was found to be located in the cytoplasmic membrane of the organism. During the cell cultivation, the oxygen demand is extremely high because *Gluconobacter* strains prefer a respiratory mode rather than a fermentative mode of growth [9]. In addition, QMEDH also requires oxygen to accomplish the oxidative reaction of meso-erythritol to L-erythrulose. Thus, the supply of oxygen to *Gluconobacter* strains is one of the most crucial factors for L-erythrulose production in an industrial process. In aerobic bioprocesses, oxygen is a key parameter; due to its low solubility in broths, a continuous supply is needed. The oxygen transfer rate (OTR)

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must be known, and if possible predicted to achieve an optimum design operation and scale-up of bioreactors [10]. Some researchers have demonstrated that baffles in flasks could enhance the agitation and increase the available surface area for oxygen transfer at the air-liquid interface [11,12,13] and thus baffled flasks have higher oxygen transfer coefficient (k_La) than unbaffled flasks under the identical shaking speed.

This paper reported, for the first time, the effects of different gradient oxygen transfer coefficient on L-erythrulose production by a newly isolated strain *G. kondonii* CGMCC8391. Based on the fermentation results of baffled flask, unbaffled flask, and fed-batch culture, a two-stage oxygen supply control strategy was investigated to improve the concentration, yield, and productivity of L-erythrulose from meso-erythritol.

2. Materials and methods

2.1. Isolation of meso-erythritol L-utilizing microorganism

Screening plates and fermentation screening medium were all prepared by meso-erythritol to screen these microorganisms which can grow in screening plates and produce L-erythrulose from meso-erythritol in this study. Samples were collected at various locations in Kaifeng (PR China), which contained sewage, sludge, rotten fruits, rotten vegetables, soil, honey, etc. All samples were stored at 4°C before isolation; 10 g of each sample was suspended in 90 mL of sterile distilled water and shaking for an hour at 30°C with 100 rpm, respectively. Aliquots of the cultures (0.2 mL) were spread on screening agar plates (100 g/L meso-erythritol, 15 g/L yeast extract, 3 g/L KH_2PO_4, 20 g/L agar, pH 6.0) and incubated at 30°C for 3 d. Strains utilizing meso-erythritol were selected from agar plates and pure cultures were obtained by slant culture. An overnight culture of isolated strains (2% inocula) was inoculated into 250 mL shake-flasks with 30 mL of fermentation screening medium (80 g/L meso-erythritol, 15 g/L yeast extract, 3 g/L KH_2PO_4, pH 6.0) and the flasks were incubated at 220 rpm shaking under 30°C for 48 h. The cultures were collected and subjected for further assay by measuring the concentration of L-erythrulose.

2.2. Microbial identification

The 16S rRNA gene was amplified from genomic DNA by PCR using the bacterial primers. The sequences of the primers used for amplification were 5′-AGAGTTTGATCATGGCTCAG-3′ (forward) and 5′-AAGGAGGTGATCCAGCCGCA-3′ (reverse), and the PCR product was purified and the sequence was determined by TaKaRa Biotechnology (Dalian) Co., Ltd. The sequence was aligned with reference sequences obtained from databases using ClustalW program. Pairwise evolutionary distances of them were calculated using Kimura's two-parameter model. A phylogenetic tree from distance matrices was constructed by the neighbor-joining method.

2.3. Physiological and biochemical characterization of strain HD385

To investigate the physiological and biochemical characteristics, standard techniques were performed, including Gram staining, the oxidase reaction, catalase, production of water-soluble brown pigment, production of L-erythrulose and 5-keto-D-gluconic acid, acid production from carbohydrates, the G + C (mol%) of L-erythrulose and etc.

2.4. Culture conditions

The culture preserved in the glycerol tube was inoculated into a 250 mL flask with 50 mL medium. After two generation cultivation, the preculture was inoculated into a shake flask (250 mL) fermentation medium with an inoculum size of 5% and cultured in an orbital shaker at 220 rpm and 30°C. Seed medium: 5 g/L yeast extract, 10 g/L peptone and 3.0 g/L KH_2PO_4 in distilled water, pH was adjusted to 6.0. Fermentation medium: 100 g/L meso-erythritol, 15 g/L peptone, 1.0 g/L KH_2PO_4 and 3 g/L $CaCO_3$ in distilled water, and the pH of the medium was adjusted to 6.0 with 2 mol/L NaOH.

2.5. Fermentation in baffled and unbaffled flasks

Meso-erythritol concentration gradient (80 g/L, 100 g/L, 120 g/L, 140 g/L and 160 g/L) was used in the fermentation medium of both baffled and unbaffled flasks in order to compare the effects of k_La on meso-erythritol translation, cell growth and L-erythrulose production. The samples were taken from each flask every 4 h during cultivation process to determine the residual meso-erythritol concentration and biomass concentration respectively. When meso-erythritol was consumed to lower than 5 g/L in the flasks, the cells were harvested and the dry cell weight (DCW) and L-erythrulose concentration were analyzed.

2.6. Fed-batch fermentations in bioreactors

Fed-batch cultures were carried out in a 5 L bioreactor BioTECH-5BG-7000A (Baoxing, China) and a 30 L NBS Bioflo 4500 (USA). The components of initial fermentation medium were the same as that of flasks, except that meso-erythritol was 100 g/L. The inoculum volume was 5% (*v*/*v*) of initial fermentation medium. The feeding solution contained 15% (*w*/*v*) meso-erythritol dissolved in tap water. The continuous feedback control strategy was employed in the fed-batch cultures, during which the feeding rate was adjusted every 4 h to maintain the residual meso-erythritol concentration in fermentation medium at 5–20 g/L with intermittent meso-erythritol feeding; the feeding rate of the next 4 h period was predicted according to the meso-erythritol consumption rate of current 4 h period and the residual meso-erythritol concentration. Furthermore, a two-stage oxygen supply control strategy was set in the fed-batch culture to provide for various k_La in different periods of the bacteria.

The cultivation temperature was kept at 30°C and pH was not controlled in the process of fermentation. DO was detected with a polarographic electrode and was expressed as percentage of O_2 saturation. 10 mL of fermentation broth was taken every 4 h for analysis of residual meso-erythritol concentration, the reaction rate and the L-erythrulose concentration. In addition, 50 mL sample was taken every 12 h to determine dry cell weight.

The air flow rate and agitation speed were set at different values to reach the varied k_La: 21.47 h^{-1} (300 rpm, 2 L/min, 5 l bioreactor), 40.28 h^{-1} (450 rpm, 3.5 L/min, 5 L bioreactor), and 86.31 h^{-1} (600 rpm, 2.5 m^3/h, 30 L bioreactor, and the pressure was controlled at 0.05 MP).

2.7. Measurement of DCW

In this study, DCW was determined with optical density of the cell [14]. Fermented liquid will be diluted multiples, then the UV absorption values was measured with spectrophotometer at 600 nm. Transferring 50 mL cell suspension to a pre-weighted centrifuge tube and centrifuged at 8000 × g for 15 min. The cell pellet was then washed twice with distillated water, and dried at 6°C for 12 h until the weight of the cell pellet does not change, then the weight is the DCW and the relationship between OD_{600} and DCW was obtained.

2.8. Meso-erythritol and L-erythrulose concentration analysis

Using HPLC method for determination of meso-erythritol and L-erythrulose concentration in fermentation broth [15]. The

chromatograph conditions were as follows: Aminex HPX-87C column (300 mm × 7.8 mm, 9 μm; Bio-Rad Chemical Division, Richmond, Calif.) with the column temperature of 60°C, acetonitrile-water (70:30 *v*/*v*) as mobile phase with the flow rate of 0.6 mL/min. L-Erythrulose and meso-erythritol were detected by a refractive index detector. L-Erythrulose yield was defined as the amount of L-erythrulose produced from 1 g initial meso-erythritol, expressed in percentage, and was corrected in accordance with the dilution factor of base or concentrated meso-erythritol solution (used in fed batch fermentation).

2.9. Measurement of oxygen transfer coefficient, k_La

The sulfite method (Na_2SO_3 method) was used for the measurement of oxygen transfer coefficient (k_La), in various cultures [16,17]. All above measurements (DCW, meso-erythritol and L-erythrulose concentration) were triplicate and recorded the average value with standard deviations.

3. Results and discussions

3.1. Screening of microorganisms producing L-erythrulose from meso-erythritol

Among about 260 samples tested, 30 microbial strains were isolated based on their abilities to grow on meso-erythritol as a sole carbon source. Through the production of L-erythrulose analysis for these candidates by HPLC, strain HD385 isolated from rotted vegetables of Kaifeng (PR China) was finally selected for its highest L-erythrulose production (64.9 g/L). The 16S rRNA gene sequence (1398 bases, GenBank accession number KJ130330) was compared with the sequence data in GenBank database by using the BLAST algorithm. According to the BLAST analysis on sequence similarity, the phylogenetic analysis was carried out by the neighbor-joining method (Fig. 1). It was shown that the closest relatives of the strain HD385 were *Gluconobacter kondonii* NBRC 3266 (100%), *Gluconobacter albidus* NBRC 3250 (99.93%), *Gluconobacter sphaericus* NBRC 12467 (99.86%), *Gluconobacter oxydanys* NBRC 14819 (99.21%). Furthermore, physiological and biochemical characterizations of strain HD385 were investigated and compared with those of the other related *Gluconobacter* species (date not shown). According to its phenotypic characteristics, strain HD385 is apparently closer to *G. kondonii* than to other *Gluconobacter* species. On the basis of sequence similarity as well as physiological, and biochemical analysis, it was concluded that newly isolated bacterial strain HD385 belongs to *G. kondonii*. This strain was deposited in China General Microbiological Culture Collection Center with the accession number of CGMCC8391. We propose the name *G. kondonii* CGMCC8391 for strain HD385.

3.2. Effects of different k_La on L-erythrulose fermentation in shake flask cultures

Some researchers have demonstrated that baffles in flasks could enhance the agitation and increase the available surface area for oxygen transfer at the air–liquid interface [11] and thus baffled flasks have higher k_La than unbaffled flasks under the identical shaking speed.

In this work, the k_La in baffled and unbaffled flasks were 54.05 h^{-1} and 23.32 h^{-1}, respectively. The difference of k_La value had lead to different performance of meso-erythritol assimilation, cell growth, and L-erythrulose production (Table 1 and Table 2). In unbaffled flask cultures, the reaction time was 20 to 40 h longer than that for the baffled flask cultures with identical meso-erythritol concentration, and the fermentation was coercive which ended at 88 h when the initial meso-erythritol concentration was higher than 100 g/L. With the increase of initial meso-erythritol concentration, the DCW did not show a continuous increase. Obviously, when the initial meso-erythritol concentration was higher than 100 g/L, the cell growth was inhibited in unbaffled flasks, which also happened in previous studies on *Schizochytrium limacinum* SR21 [18,19,20]. In the baffled flask cultures, meso-erythritol was completely exhausted after shorter fermentation time at different initial concentrations.

In the unbaffled flasks, L-erythrulose yield almost decreased from 0.84 g/g to 0.63 g/g with the increase of initial meso-erythritol concentrations from 80 g/L to 160 g/L. However, in the baffled flasks, much higher yield was achieved (0.89–0.92 g/g). DCW and L-erythrulose concentration in the baffled flask cultures were higher than those in unbaffled flasks, which confirmed the previous statement that oxygen condition could influence the cell growth, yield and L-erythrulose accumulation [21,22].

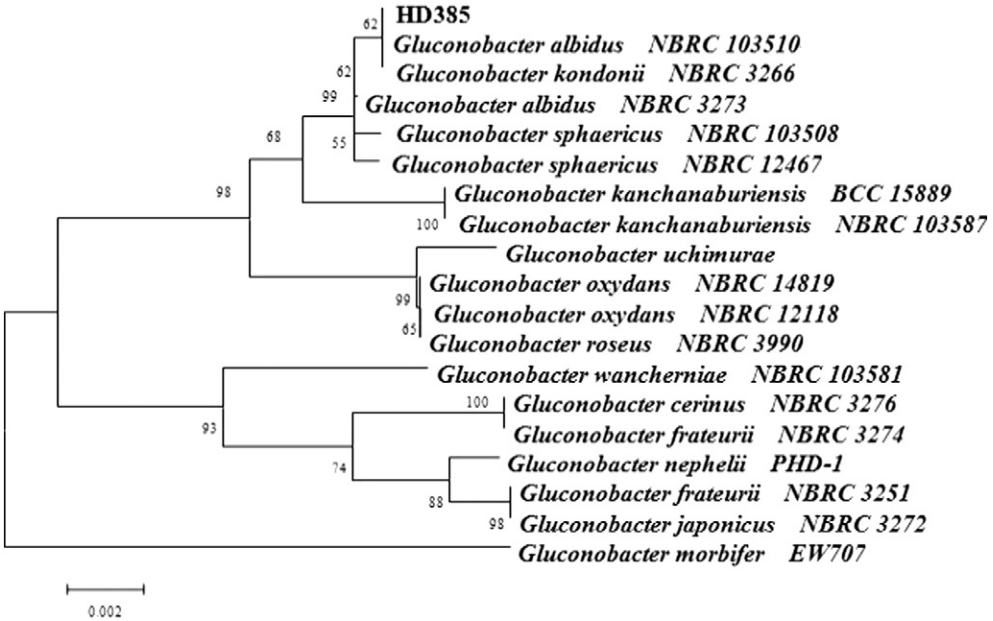

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences similarity of strain HD385 and the reference strains.

Table 1
The effect of initial meso-erythritol concentration on cell growth and L-erythrulose production in unbaffled flasks culture by *G. kondonii* CGMCC8391 (k_La, 23.32 h^{-1}).

Initial meso-erythritol (g/L)	Fermentation time (h)	DCW (g/L)	L-Erythrulose concentration (g/L)	Yield (g/g)	Q_p (g/L/h)
80	44	1.63 ± 0.13	67.5 ± 2.35	0.84	1.53
100	56	1.65 ± 0.14	83.8 ± 3.27	0.84	1.50
120	88[a]	1.43 ± 0.08	101.5 ± 3.61	0.84	1.15
140	88[a]	1.36 ± 0.11	104.0 ± 4.63	0.74	1.18
160	88[a]	1.27 ± 0.09	101.0 ± 4.08	0.63	1.15

The data are the average value of triplicate measurements with standard deviations.
[a] Erythritol concentration of 120 g/L, 140 g/L and 160 g/L fermentation, the obtained data were measured in 88 h fermentation, meso-erythritol was still not completely consumed at this time.

For the baffled flask with an initial meso-erythritol concentration of 160 g/L, the L-erythrulose concentration was achieved at as high as 147.6 g/L. In this study, it took only 44 h to deplete 160 g/L meso-erythritol in baffled flasks, and furthermore, a significantly higher L-erythrulose productivity (3.35 g/L/h) was obtained.

In the shake cultures, available oxygen was assumed to be limiting since the only source was surface aeration. Since the role of k_La on cell growth, meso-erythritol utilization and L-erythrulose production was so important in the flask cultures that encouraged us to design even higher k_La values in larger bioreactors to achieve even better results.

3.3. Effects of different constantly k_La on fed-batch fermentation

In aerobic fermentation process, the solubility of oxygen in aqueous solutions at ambient temperature and pressure is only about 10 ppm, which is usually quickly consumed in aerobic cultures. Thus, it is of primary importance that dissolved oxygen (DO) must be constantly supplied and transferred at high rate from gas bubbles to cell clumps for intracellular reaction. The effects of DO concentration on dihydroxyacetone (DHA) production by *Gluconobacter* strains and the application of a DO control strategy to DHA production have been studied by several research groups [23,24]. However, DO control is not very practical in industrialization, because it varies with agitation speed, cell concentration, carbon source feeding rate and concentration, bioreactor size etc. Alternatively, the k_La can be considered a promising strategy for improving the supply and transfer of oxygen in vivo because it has been correlated with the combination of stirrer speed, superficial gas velocity and liquid effective viscosity, which are measurable scaling-up parameters.

The results from flask cultures have showed that the cell growth and L-erythrulose production were inhibited when the initial meso-erythritol concentration was above 100 g/L. Therefore, in the fed-batch cultures, the initial meso-erythritol concentration was set at 100 g/L to prevent the inhibition phenomenon from occurring in bioreactors, and meso-erythritol concentration was controlled at 5–20 g/L.

When the low k_La was employed (21.47 h^{-1} in 5 l bioreactor), DO decreased from 100% to less than 1% at 16 h and then was almost kept at 0–3%. Oxygen molecules could not effectively overcome a series of transport resistance, which led to a low dissolved oxygen level in culture medium during the exponential growth phase, and thus caused the weak metabolic activities. As a consequence, cell growth was limited by the low dissolved oxygen levels, and only small amount of substrate was consumed and a low biomass concentration was achieved (DCW, 1.87 ± 0.26 g/L). As is shown in Fig. 2, the fermentation process was ended at 60 h because the consumption rates of meso-erythritol were very slow, which was presumably due to the weak metabolic activity of cells under low dissolved oxygen. Consequently, the L-erythrulose concentration, yield and productivity were only 82.0 ± 3.68 g/L, 0.82 g/g, and 1.37 g/L/h, respectively.

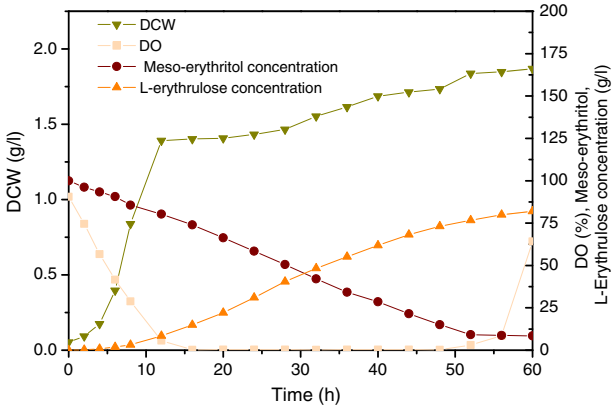

Fig. 2. Time course of fermentation profiles of *G. kondonii* CGMCC8391 in fed-batch culture on meso-erythritol with k_La at 21.47 h^{-1}. Experiments were carried out in three trials. The figures gave the results of the average value of triplicate measurements.

When k_La was at a higher value of 40.28 h^{-1} (Fig. 3), substrate assimilation was obviously activated during which DO decreased from 100% to less than 10% at 12 h and then was almost kept at 0–10%. During the exponential growth phase, the DO value maintained an appropriate level, which led to a well cell growth (DCW was 2.61 ± 0.19 g/L) because the oxygen inhibition was eliminated. At the end of fermentation (48 h), the L-erythrulose concentration, yield and productivity increased to 140.6 ± 4.53 g/L, 0.87 g/g, and 2.93 g/L/h, respectively.

When k_La was elevated to 86.31 h^{-1}, more DO was supplied during all the fermentation process. As it was shown in Fig. 4, DO rapidly decreased to 46.5% at 8 h and then it was almost maintained above 40%. Though the oxygen supply was more sufficient compared with that in the middle k_La, a slightly lower DCW was achieved (2.20 ± 0.34 g/L). The key enzyme (QMEDH), which is responsible for

Table 2
The effect of initial meso-erythritol concentration on cell growth and L-erythrulose production in baffled flasks by *G. kondonii* CGMCC8391 (k_La, 54.05 h^{-1}).

Initial meso-erythritol (g/L)	Fermentation time (h)	DCW (g/L)	L-erythrulose concentration (g/L)	Yiel (g/g)	Q_p (g/L/h)
80	20	2.21 ± 0.27	71.8 ± 2.41	0.89	3.59
100	24	2.18 ± 0.19	92.3 ± 3.72	0.92	3.85
120	28	1.87 ± 0.21	106.3 ± 3.94	0.89	3.80
140	36	1.75 ± 0.23	127.3 ± 4.47	0.91	3.54
160	44	1.59 ± 0.17	147.6 ± 4.77	0.92	3.35

The data are the average value of triplicate measurements with standard deviations.

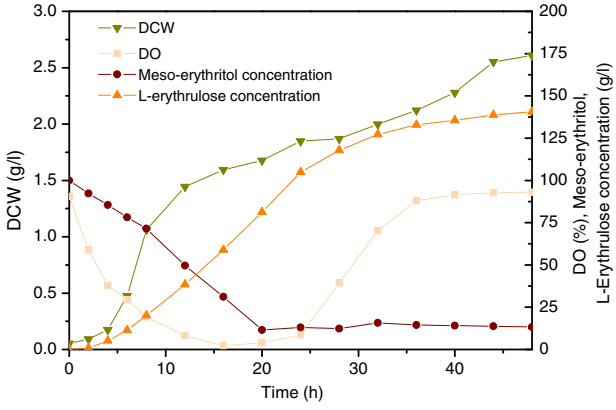

Fig. 3. Time course of fermentation profiles of *G. kondonii* CGMCC8391 in fed-batch culture on meso-erythritol with k_La at 40.28 h^{-1}. Experiments were carried out in three trials. The figures gave the results of the average value of triplicate measurements.

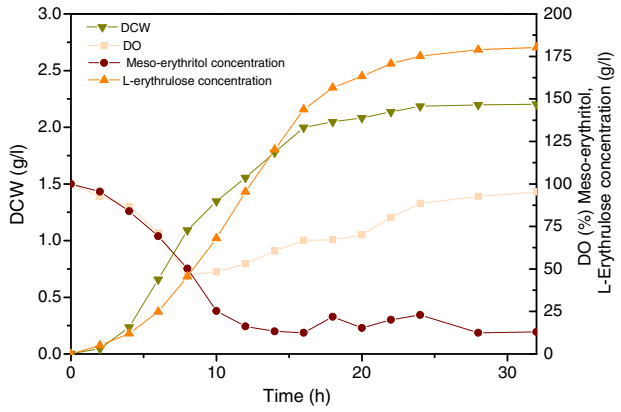

Fig. 4. Time course of fermentation profiles of *G. kondonii* CGMCC8391 in fed-batch culture on meso-erythritol with k_La at 86.31 h^{-1}. Experiments were carried out in three trials. The figures gave the results of the average value of triplicate measurements.

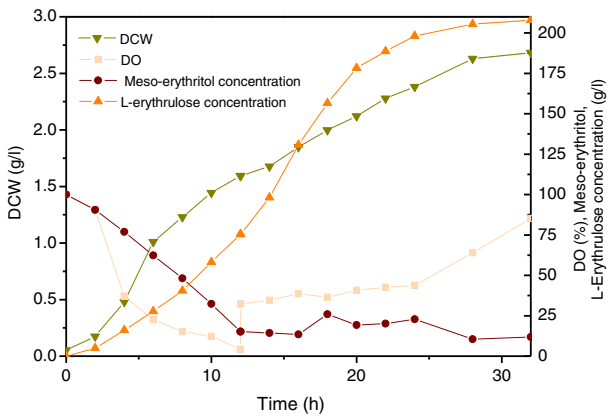

Fig. 5. Time course of fermentation profiles of *G. kondonii* CGMCC8391 in fed-batch culture on meso-erythritol with a two-stage oxygen supply control strategy. Experiments were carried out in three trials. The figures gave the results of the average value of triplicate measurements.

L-erythrulose production, requires oxygen to accomplish the oxidative reaction of meso-erythritol to L-erythrulose. Therefore, the highest L-erythrulose concentration (180.3 g/L) was achieved at the end of fermentation (32 h) with the high k_La (86.31 h^{-1}) during the three fed-batch cultures, and the L-erythrulose yield and productivity increased to 0.91 g/g and 5.63 g/L/h, respectively.

As shown in Table 3, the results of the three fed-batch cultures showed that k_La significantly affected the substrate assimilation, cell growth and L-erythrulose production. The constantly high k_La brings a high dissolved oxygen concentration during the fermentation, leading to an increase in the cell growth, substrate assimilation, and L-erythrulose production.

3.4. A two-stage oxygen supply control strategy

In shake flask culture and fed-batch culture of bioreactors, it has been proven that the supply of oxygen was necessary during the fermentation process with *G. kondonii* CGMCC8391. However, different from L-erythrulose concentration, the maximum DCW value was not achieved in the highest k_La (86.31 h^{-1}). When the fermentation was under the k_La of 21.47 h^{-1}, the dissolved oxygen was so low that it limited the growth of the cell. But when the value of k_La came to 86.31 h^{-1}, the agitation rate was probably too high that the cell was exposure to high share rates which caused the cell deformation and damage suffered growth of bacteria [18]. It can be concluded that the cell growth and L-erythrulose production could not be achieved simultaneously by controlling a constant k_La throughout the whole culture process. As demonstrated in a batch culture of *S. limacinum*, a shift process was devised to provide an appropriate DO environment to improve the production of docosahexaenoic acid [25]. Since cell growth required appropriate DO while L-erythrulose accumulation required high DO, a shift process was devised to provide a favorable DO environment. Therefore, in this study, an oxygen supply control strategy was proposed to enhance the production of L-erythrulose in the fed-batch culture based on the analysis described above.

Fig. 5 depicted the fermentation profiles of *G. kondonii* CGMCC8391 in fed-batch culture on meso-erythritol with a two-stage oxygen supply control strategy. During the first 12 h, the k_La was set at 40.28 h^{-1}, and then it was kept at 86.31 h^{-1} after 12 h. At the first stage, cell grew faster than that in high k_La (Fig. 4), and the DCW reached to 2.68 g/L at 12 h. After 12 h, the DO had a rapid increase and then maintained above 40% because the k_La elevated to 86.31 h^{-1}. At the end of fermentation (32 h), the L-erythrulose concentration, yield and productivity increased to 207.9 ± 7.78 g/L, 0.94 g/g, and 6.50 g/L/h, respectively, which were higher than those in other three cultures.

Table 3 showed the contrast of L-erythrulose production in fed cultures at four different k_La. It can be concluded that two-stage oxygen supply control strategy could not only considerably improve the DCW but also increase the L-erythrulose concentration and productivity. In this strategy, the k_La was controlled at 40.28 h^{-1} in the first 12 h for fast cell growth, and the max DCW (2.68 ± 0.17 g/L) was obtained, which was higher than that in other three cultures. In the second stage with the k_La of 86.31 h^{-1}, more dissolved oxygen was supplied during the fermentation process, leading to more L-erythrulose transformed from meso-erythritol. The reason was that QMEDH (the key enzyme in the L-erythrulose formation pathway) obtained abundant oxygen to accomplish the oxidative under high k_La. In this study, the proposed two-stage oxygen supply control strategy was proven to be effective for efficient L-erythrulose production. Compared with the previously reported, the maximum L-erythrulose concentration (207.9 ± 7.78 g/L) and productivity (6.50 g/L/h) achieved during the above fed batch fermentation were higher than those in the batch fermentation.

4. Conclusions

A newly strain producing L-erythrulose from meso-erythritol was isolated from rotted vegetables and conserved in the laboratory. A series of morphological and biochemical characteristics and sequence

Table 3
The results of L-erythrulose production by *G. kondonii* CGMCC8391 in fed-batch cultures at different k_La.

k_La	21.47 h^{-1}	40.28 h^{-1}	86.31 h^{-1}	40.28 h^{-1} (0–12 h), 86.31 h^{-1} (after 12 h)
Fermentation time (h)	60	48	32	32
DCW (g/L)	1.87 ± 0.26	2.61 ± 0.19	2.20 ± 0.34	2.68 ± 0.17
L-Erythrulose concentration (g/L)	82.0 ± 3.68	140.6 ± 4.53	180.3 ± 5.60	207.9 ± 7.78
Yield (g/g)	0.82	0.87	0.91	0.94
Q_p (g/L/h)	1.37	2.93	5.63	6.50

The data are the average value of triplicate measurements with standard deviations.

analysis of 16S rRNA revealed that it belonged to *G. kondonii*. During the fed batch fermentation in a 30-L stirred bioreactor with two-stage oxygen supply control strategy, the L-erythrulose concentration (207.9 g/L) and productivity (6.50 g/L/h) were higher than those in the previous similar reports.

In conclusion, meso-erythritol could be efficiently used for the L-erythrulose production by *G. kondonii* strains. The present study should be potentially useful for the efficient L-erythrulose production on industrial scale.

Conflict of interest

There is no conflict of interest.

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