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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.

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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

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