BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Industrial Microbiology

Effects of oxygen transfer coefficient on dihydroxyacetone production from crude glycerol

Xiao-juan Zheng[◊], *Kui-qi Jin*[◊], *Lei Zhang*, *Gang Wang*, *Yu-Peng Liu*[*]

Institute of Bioengineering, School of Life Sciences, Henan University, Kaifeng 475004, PR China

ARTICLE INFO

Article history:
Received 3 December 2014
Accepted 9 July 2015

Associate Editor: Jorge Gonzalo Farias Avendano

Keywords:
Dihydroxyacetone
k_La
Biodiesel-derived
Crude glycerol
Gluconobacter frateurii

ABSTRACT

The principal objective of this study was to evaluate the kinetics of dihydroxyacetone production by *Gluconobacter frateurii* CGMCC 5397 under different oxygen volumetric mass transfer coefficient (k_La) conditions in submerged bioreactors using biodiesel-derived crude glycerol as the carbon source. k_La is a key fermentation parameter for the production of dihydroxyacetone. Cultivations were conducted in baffled- and unbaffled-flask cultures (the k_La values were 24.32 h^{-1} and 52.05 h^{-1}, respectively) and fed-batch cultures (the k_La values were held at 18.21 h^{-1}, 46.03 h^{-1}, and 82.14 h^{-1}) to achieve high dihydroxyacetone concentration and productivity. The results showed that a high k_La could dramatically increase dihydroxyacetone concentrations and productivities. The baffled-flask culture (with a k_La of 52.05 h^{-1}) favored glycerol utilization and dihydroxyacetone production, and a dihydroxyacetone concentration as high as 131.16 g/L was achieved. When the k_La was set to 82.14 h^{-1} in the fed-batch culture, the dihydroxyacetone concentration, productivity and yield were 175.44 g/L, 7.96 g/L/h and 0.89 g/g, respectively, all of which were significantly higher than those in previous studies and will benefit dihydroxyacetone industrial production.

Introduction

Dihydroxyacetone (abbreviated as DHA) is a commercially important chemical used in the cosmetic industry as a self-tanning agent.[1,2] DHA has also been proposed to be involved in weight augmentation and fat loss, antioxidant activity, and increasing endurance capacity,[3] and it offers a safe and effective therapeutic option for recalcitrant vitiligo. Additionally, DHA is an important precursor for the synthesis of various fine chemicals and pharmaceuticals and serves as a versatile building block for the organic synthesis of a variety of fine chemicals.[4]

Crude glycerol, an important chemical, is abundantly available because it is the main byproduct of the conversion of vegetable oils into biodiesel. The progressive increase in biodiesel production has in turn caused a sudden increase in crude glycerol. Approximately 4.53 kg of crude glycerol are created for every 45.3 kg of biodiesel produced. The purification of crude glycerol to a chemically pure substance results in a valuable industrial chemical. However, purification is costly, and the glycerol market is already saturated. Thus,

* *Corresponding author.*
E-mail: liuyupenglw@126.com (Y.-P. Liu).
◊ These authors contributed equally to this work.
http://dx.doi.org/10.1016/j.bjm.2015.11.020

Nomenclature

X_{max}	maximum cell concentration (g/L)
P_{max}	maximum production (DHA) concentration (g/L)
T	fermentation time to reach the maximum production of DHA (h)
μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})
Q_p	DHA productivity (g/L/h)

the price of crude glycerol continues to decline and directly affects biodiesel production costs.[5] Therefore, it is essential to discover new applications for crude glycerol that will provide an ideal platform for chemical and pharmaceutical industries. Some value-added chemicals produced from crude glycerol have been developed, such as epichlorohydrin, DHA, 1,3-propanediol, propionic acid, polyhydroxyalkanoate,[6] erythritol,[7] citric acid,[8] hydrogen,[9] mannitol,[10] lipids,[11] mixed acids,[12] and eicosapentaenoic acid.[13]

Since Bertrand first observed the production of DHA from glycerol by bacteria in 1898, various microorganisms used for DHA production have been reported.[14] *Gluconobacter* strains produce DHA via the incomplete oxidation of glycerol with membrane-bound glycerol dehydrogenases (GDHs)[15] and are the most extensively used microorganisms for DHA production.[16,17] During cell growth, oxygen demand is extremely high because *Gluconobacter* strains prefer a respiratory, rather than a fermentative, mode of growth.[18] In addition, GDH also requires oxygen to accomplish the oxidative conversion of glycerol to DHA.[19] Thus, the supply of oxygen to *Gluconobacter* strains is one of the most crucial factors for DHA production in an industrial process.

Various fermentation strategies have been previously reported to enhance oxygen supply during the DHA production process. Hydrogen peroxide and p-benzoquinone have been added to media as an oxygen source and an electron acceptor.[20] Oxygen carriers such as hemoglobin, perfluorochemicals, and silicone oils have also been used to enhance oxygen supply in fermentation media.[21] Moreover, some conventional techniques to improve the rate of oxygen transfer from the gas phase have been investigated, including increasing the agitation or aeration rate, raising the partial pressure of oxygen in the gas phase or modifying the bioreactor design.[4]

The effects of different oxygen supply conditions can be measured by the oxygen volumetric mass transfer coefficient (k_La). The oxygen volumetric mass transfer coefficient is generally considered to be a critical parameter in aerobic cultures of microorganisms due to its poor solubility and the need for a constant supply. The selection, design, and scale-up of biochemical reactors and the accurate estimation of mass transfer rates for different scales and different operational conditions are of critical importance in bioprocesses.[22] As with any other transport phenomena occurring between different phases, the oxygen transfer rate is profoundly affected by hydrodynamic conditions in the bioreactor, which affect the mass transfer coefficient and the interfacial area. Therefore, variations in the configurations of bioreactors and the physicochemical properties of the culture medium, including density, diffusivity, rheological properties, and surface tension, can attenuate the oxygen transfer capacity, resulting in oxygen depletion.[23]

Previously, a newly isolated strain, *Gluconobacter frateurii* CGMCC 5397, was shown to utilize inexpensive biodiesel-derived crude glycerol to produce DHA.[24] However, in our previous study, DHA concentration and productivity were not satisfactory. In this study, the effect of the oxygen transfer coefficient on DHA production from biodiesel-derived crude glycerol by *Gluconobacter frateurii* was investigated to enhance DHA concentration and productivity. Both baffled- and unbaffled-flask cultures were used in the fermentation process, and fed-batch cultures with three different k_La values were also compared.

Materials and methods

Microorganism

The strain *Gluconobacter frateurii* CGMCC 5397, isolated from rotting fruits using a crude glycerol culture in our laboratory, was employed in this work, stored at 4 °C on GY agar slants, and transferred monthly. The medium (GY) consisted of 25 g/L glycerol, 5 g/L yeast extract, and 20 g/L agar.

Crude glycerol

Crude glycerol used in this work was obtained from a palm oil-based biodiesel plant in Malaysia operated by Vance Bioenergy [composition: 80.5% (w/w) glycerol, 10.1% (w/w) water, 5.2% (w/w) sodium salts, 0.4% (w/w) potassium salts, 0.3% (w/w) other salts, 0.5% (w/w) methanol, and 2% (w/w) other organics (esters, free fatty acids, soaps, etc.)]. Crude glycerol was used as a carbon source in the culture medium without purification. The purity of the crude glycerol (80.5%, w/w) used in each experiment was taken into consideration when making the appropriate calculations; thus, the initial concentrations of glycerol quoted refer to pure glycerol.

Shake flask cultures

A crude glycerol concentration gradient (50 g/L, 75 g/L, 100 g/L, 125 g/L and 150 g/L) was used in the fermentation media of both the baffled and unbaffled flasks to compare the effects of k_La on glycerol oxidation, cell growth and DHA production. The other fermentation medium components included 24 g/L corn steep liquor and 3 g/L $CaCO_3$. The seed medium contained 15 g/L crude glycerol, 15 g/L yeast extract, and 3 g/L KH_2PO_4. The pH of the medium was adjusted to 6.0 with 2 M NaOH, and the medium was heat sterilized (20 min at 121 °C). The seed medium was inoculated with a 2 mL stock culture stored at −80 °C and incubated at 30 °C shaking at 200 rpm for 16 h. For the fermentation experiments, the medium was inoculated with 5% of the seed culture and incubated shaking at 200 rpm at 30 °C for 48 h. The cells were grown in 250-mL flasks with a working volume of 50 mL.

During the cultivation process, 1-mL samples were taken from each flask every 8 h to determine the residual glycerol