Bioprocess Biosyst Eng (2016) 39:1315–1318
DOI 10.1007/s00449-016-1595-8

SHORT COMMUNICATION

Improving the production yield and productivity of 1,3-dihydroxyacetone from glycerol fermentation using *Gluconobacter oxydans* NL71 in a compressed oxygen supply-sealed and stirred tank reactor (COS-SSTR)

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Received: 13 December 2015 / Accepted: 17 March 2016 / Published online: 28 March 2016

Abstract In this study, a compressed oxygen gas supply was connected to a sealed aerated stirred tank reactor (COS-SSTR) bio-system, leading to a high-oxygen pressure bioreactor used to improve the bio-transformative performance in the production of 1,3-dihydroxyacetone (DHA) from glycerol using *Gluconobacter oxydans* NL71. A concentration of 301.2 ± 8.2 g L^{-1} DHA was obtained from glycerol after 32 h of fed-batch fermentation in the COS-SSTR system. The volumetric productivity for this process was 9.41 ± 0.23 g L^{-1} h^{-1}, which is presently the highest obtained level of glycerol bioconversion into DHA. These results show that the application of this bioreactor would enable microbial production of DHA from glycerol at the industrial scale.

Keywords 1,3-Dihydroxyacetone (DHA) · Glycerol · COS-SSTR · *Gluconobacter oxydans* NL71

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Introduction

Glycerol, a promising and abundant carbon source for industrial microbiology applications, is also an important platform chemical compound because of its role as a structural component of many lipids [1]. In recent years, biodiesel has garnered considerable interest as a viable fuel and fossil diesel additive. Due to the rapid increase in biodiesel production during the recent years, there is now an over-abundance of glycerol as main by-product of biodiesel manufacturing [2]. Thus, transformative technological developments and applications for glycerol are sorely needed. 1,3-dihydroxyacetone (DHA), synthesized from glycerol, is one kind of value-added chemical, the production of which would be very useful. Aside from its extensive use in the food industry, in cosmetics, and in the pharmaceutical industry, DHA is also an important platform chemical compound for organic synthesis [3–5]. DHA can be produced using either chemical synthesis or bio-conversion. Notably, microbial bio-transformation of DHA has been found to be more environmentally friendly and cost-efficient than chemical methods of DHA production [6].

Some studies have reported the DHA production from glycerol using *Gluconobacter oxydans* (*G. oxydans*). Moreover, *G. oxydans* has become one of the most frequently used strains of bacteria in the industrial production of DHA [7–9]. However, until now the desired DHA yield has not been achieved because the high concentrations of glycerol can inhibit microbe growth and thus DHA synthesis [10, 11]. Although the fed-batch bio-process has been widely applied in bio-conversion of DHA as it is able to alleviate glycerol inhibition to some extent, the negative effect of the excess amount of glycerol on the DHA production has been unavoidable until today.

DHA can be used as a carbon source in glycerol-limited medium and the resulting delay in reaction time would cause a decrease in the DHA production [12]. Since the production of DHA from the fermentation of glycerol by *G. oxydans* requires high oxygen, the DHA yield and productivity of this process could be improved by a controlled dissolved oxygen (DO) strategy [13–15].

We have successfully established in a previous work a COS-SSTR system that included a whole cell catalysis of the isolated strain *G. oxydans* NL71. This system markedly increased DO and permitted to obtain high xylonic acid yield and volumetric productivity [16, 17]. The microbial catalytic mode of DHA is similar to that of xylonic acid, in which the glycerol fermentive procedure is also a close-coupling oxidation reaction comprised of dehydrogenation and a cellular respiration chain which is heavily dependent on a sufficiently ample supply of oxygen [18, 19]. In other words, oxygen is the final driving force which runs the serial oxidation reactions that render the catalysis of glycerol to produce DHA in a continuous conversion process. Likewise, the design of a COS-SSTR could theoretically be applied to the DHA bio-production.

In this study, the performance of fermentation was tested in an aerated stirred tank reactor (ASTR) and in a COS-SSTR. The results obtained from each reactor were then compared. The objective of this work is to improve the output and productivity of the DHA bio-transformation using COS-SSTR in order to provide a feasible technology for industrial production of DHA from glycerol.

Materials and methods

Microorganism

Gluconobacter oxydans NL71 were isolated from the strain of *G. oxydans* ATCC 621 and were maintained in a sorbitol-agar colony (sorbitol 50 g L^{-1}, yeast extract 5 g L^{-1}, agar 20 g L^{-1}) at 4 °C.

Media and culture conditions

The inocula of *G. oxydans* NL71 were prepared in a 250 mL Erlenmeyer shaker flask containing 50 mL of medium (sorbitol 50 g L^{-1}, yeast extract 5 g L^{-1}), and cultured for 24 h at 220 rpm and 30 °C.

The fermentation medium consisted of the following components (in g L^{-1}): sorbitol 30, yeast extract 5.0, $MgSO_4$ 0.5, K_2HPO_4 1.0, $CaCO_3$ 2, and glycerol. The inoculum ratio was 5 % (v/v). The fed-batch fermentation for DHA production was performed in a 3.0 L fermentor (New Brunswick GelliGen 115) using ASTR or COS-SSTR. Glycerol was fed at the growth anaphase of *G. oxydans* NL71. The pH was stabilized at 5.0 during the cell growth stage and at 6.0 during the fermentation stage by an automated addition of a NaOH solution (10 %). The foaming was controlled online automatically with the addition of a polyether-ether-ketone defoamer for the ASTR batches, but not in the COS-SSTR batches.

The conditions of the ASTR batches were: 1.0 L of broth, 30 °C, 500 rpm, and an airflow of 3 vvm. We did not use airflow for the COS-SSTR batches but pure oxygen which was connected to an oxygen cylinder (purity ≥99.9 %) with a gas inlet pressure of 0.02–0.05 MPa [16].

Analytical methods

DHA and glycerol levels were determined using gas chromatography according to the method described by Wang et al. [20]. The volumetric productivity (g L^{-1} h^{-1}) of DHA was calculated from the concentration of DHA divided by the reaction time (which was, in turn, calculated from the time of the fed-batch).

Two parallel assays were performed for each sample.

Results

Effect of the aeration rate on the fermentation

The transformative performance of the *G. oxydans* NL 71 was first tested in the ASTR. Since the produced DHA could be separated from the microbial fermentation liquor and purified by crystallization [21], the glycerol could be directly fed into the fermentation medium for the production of DHA. The kinetic parameters of the DHA transformation are described in Fig. 1. We obtained 198.2 ± 9.2 g L^{-1} of DHA after a fermentation duration of 102 h and the overall volumetric production was 1.94 ± 0.09 g L^{-1} h^{-1}. These results are similar to the ones found in literature [13–15].

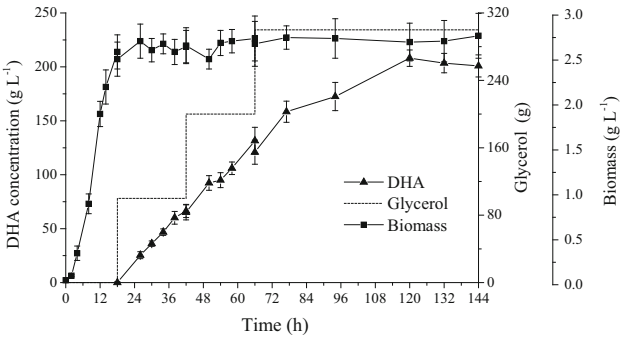

Fig. 1 Reaction process of DHA fermentation in ASTR. Recorded concentrations of DHA (*triangles*) produced and of the biomass (*squares*) as a function of time during the fermentation of glycerol into DHA in the ASTR via a fed-batch operation with three batch additions of 100 g of glycerol (*dotted curve*) at 18, 42, and 66 h

Once the DHA reached a concentration of about 200 g L^{-1}, the glycerol was no longer converted and the microbial catalysis activity reduced presumably due to the DHA toxicity. This issue is well-explained in the context of common microbial growth and metabolism in DHA medium where repressed and feeble cells can no longer catalyze glycerol at some point and remains an issue [11, 22]. Thus, the investigatory focus was to solve this problem and thereby improve the yield of DHA to enhance the efficiency of the entire process. Our objective was to use the COS-SSTR system instead of the conventional common airflow bioreactor system to significantly enhance the bioproduction process for producing DHA from glycerol.

Fermentation improved by using COS-SSTR

When processed in the COS-SSTR system, the strain of *G. oxydans* NL71 exhibited excellent biotransformative performance for the production of DHA. The dissolved oxygen (DO) level was increased substantially in the COS-SSTR in contrast to the DO levels seen in the common ASTR. Figure 2 shows that a level of 301.2 ± 8.2 g L^{-1} of DHA was obtained after a fermentation duration of 32 h. Apparently, the yield of DHA was improved by enhancing the level of the DO in the sealed stirred tank reactor. Furthermore, compressed oxygen supply benefited the cell growth (biomass) such that the overall volumetric productivity of DHA was 9.41 ± 0.23 g L^{-1} h^{-1}.

Discussion

Gluconobacter oxydans is one of the most frequently used microorganisms in the bio-production of valuable chemicals [7]. A strain named *G. oxydans* NL 71 was isolated from *G. oxydans* ATCC 621 using the crude lignocellulosic hydrolysate domestication process. *Gluconobacter oxydans* NL71 exhibited an impressive rate of catalytic oxidation of xylose into xylonic acid and a remarkable tolerance to lignocellulosic inhibitors that can result from microbial genome mutation [16, 17]. The bioconversion performance of *G. oxydans* NL71 was tested in a 3 L airflow bioreactor by fed-batch. We have chosen this process because it can lessen the inhibitory effects of substrates to a certain extent [13].

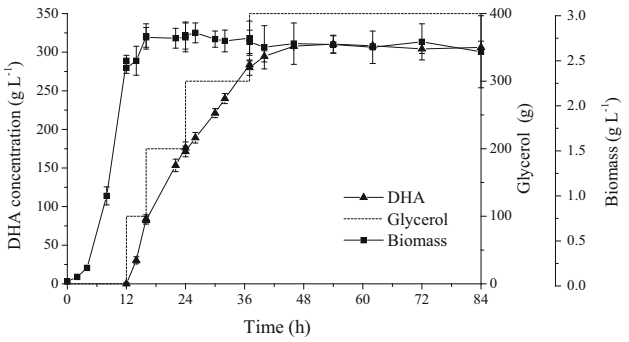

Fig. 2 Reaction process of DHA fermentation in COS-SSTR. Recorded concentrations of DHA (*triangles*) produced and of the biomass (*squares*) as a function of time during the fermentation of glycerol into DHA in the COS-SSTR via a fed-batch operation with four batch additions of 100 g of glycerol (*dotted curve*) at 12, 16, 24, and 38 h

The DHA industrial production by microbial oxidation of glycerol presents the main drawback of low productivity specifically due to the long periods of fermentation and the excessive consumption of energy. Moreover, *G. oxydans* is an obligate aerobe; accordingly, to achieve a higher yield of the desired product, the process requires an abundant oxygen supply during the oxidation of glycerol into DHA [7, 22]. During the production of DHA, the conversion rate of glycerol into DHA increased together with the application of oxygen enriched aeration [13, 15]. To date, literature reports various DO control strategies to improve the production efficiency of DHA transformation [13–15, 23]. While certain strategies have improved the yield, the results have not been entirely satisfying.

Previously Zhou et al. proposed a method of COS-SSTR to drastically improve the production efficiency of xylonic acid catalysis [16]. Based on the nature of *G. oxydans*, the reactive process observed for the conversion of glycerol as well as xylose is a close-coupling oxidation reaction system. This system is a combination of dehydrogenase family members bound to the cytoplasmic membrane and of the cellular respiration chain. This entire process depends heavily on oxygen because of the terminal electron acceptor, and there is little degradation of the product [18, 19]. Theoretically speaking, under the optimum conditions for fermentation, H_2O is the main by-product formed in this oxidation reaction and little exhaust gas is released. Thus, the strength of this design was the maintenance of closed gas outlets and the addition of an inlet for compressed pure oxygen which increased the oxygen pressure in the system.

Therefore, with the goal of improving the efficiency of DHA production, the catalytic oxidation of glycerol was investigated in the COS-SSTR. In the ASTR, in the process of conversion, the DO level always keep under the 5 %, yet, the DO level of COS-SSTR was almost 8 times than ASTR. Finally, we could find that the productivity of COS-SSTR showed an improvement of almost 4.8-fold compared to the results obtained in the ASTR (Table 1). The fermentation time was shortened to 32 h therefore reducing effectively the above-explained drawback of microbial production. In addition, during the process of conversion no foam was formed and no exhaust gas was released. Therefore, the contradiction between the high airflow and high concentration fermentation was solved. In other words, the COS-SSTR system significantly enhanced the

Table 1 Summary of the reaction parameters of DHA fed-batch fermentations in a ASTR and a COS-SSTR

Reactor type	Biomass ($g\ L^{-1}$)	Reaction time when maximum DHA concentration is reached (h)	Concentration of DHA ($g\ L^{-1}$)	Productivity of DHA ($g\ L^{-1}\ h^{-1}$)
ASTR	2.61 ± 0.12	102	198.2 ± 9.2	1.94 ± 0.09
COS-SSTR	2.75 ± 0.09	32	301.2 ± 8.2	9.41 ± 0.26

overall efficiency of fermentation and reduced the fermentation time in the sealed stirred tank reactor. The reaction stopped when the concentration of DHA reached more than about 300 $g\ L^{-1}$, meaning that the inhibitory effect of the DHA could not be completely avoided. Nevertheless, the COS-SSTR process still exhibited a significant improvement with a more rapid oxidation of glycerol for the production of DHA and using a design which is feasible for industrial-scale applications.

In conclusions, based on the nature of *G. oxydans* NL 71, an automatic oxygen-supply bioreactor was proposed to improve the bioconversion level of glycerol into DHA. The use of the COS-SSTR enhanced the bioconversion efficiency significantly. Finally, a DHA concentration of $301.2 \pm 8.2\ g\ L^{-1}$ at a volumetric productivity of $9.41 \pm 0.23\ g\ L^{-1}\ h^{-1}$ was directly produced starting from about 400 g glycerol by fed-batch. According to these results, we have improved efficient utilization of glycerol, and enable the comprehensive utilization and technical integration of biodiesel production.

Acknowledgments The research was supported by the National Natural Science Funding of China (31370573) and the Key Research and Development Program of Jiangsu (BE2015758). Also, the authors gratefully acknowledge financial support from the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) and the Doctorate Fellowship Foundation of Nanjing Forestry University for supporting the work presented in this paper.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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