International Journal of Applied Engineering Research
ISSN 0973-4562 Volume 10, Number 6 (2015) pp. 14615-14620

http://www.ripublication.com

Microbial Conversion of Glycerol To Dihydroxyacetone by Using Batch Mode

M. Acevedo-Morantes[a], G. De Ávila[a], A. Realpe-Jiménez[a]
[a] *Programa de Ingeniería Química, Facultad de Ingeniería, Universidad de Cartagena, Colombia*

Abstract

The production of dihydroxyacetone (DHA) of glycerol through microbial conversion with Gluconobacter *oxydans* was studied in batch mode for several initial concentrations of glycerol, 20, 50 and 100 g/L. Theconcentrations of glycerol and DHAand yield were determined. The DHA concentration and yield of the glycerol oxidation were dependent of initial substrate concentration. Inhibitory effect with substrate is observed at high initial concentrations of glycerol, greater than 50 g/l, since there are not changes in the DHA production between glycerol fermentations of 50 and 100 g/l.

Keywords: glycerol fermentation, inhibitory effect, dihydroxyacetone, Gluconobacter oxydans.

Introduction

Dihydroxyacetone (DHA) is a chemical product obtained industrially by glycerol oxidation via microbial conversion with *Gluconobacter oxydans*. The several applications of DHA in chemical and pharmaceutical industry promote it as significant compound with a higher market price 100 times that of glycerol [1] and a potential increase of the demand in the next years [2] considering that DHA can be used as a building block in organic synthesis of fine chemicals [3-5]and in the production of biodegradable polymers [6].

The bioconversion of glycerol to DHA by *Gluconobacter oxydans*, as eco-friendly technology, can provide high selectivity at moderate temperature with higher cost effective than the chemical synthesis [7]. However, the yield of glycerol oxidation is dependent strongly to high concentrations of substrate and product due to inhibition on cell growth [8].

The present study concerns the DHA production at three concentrations of glycerol, 20, 50 and 100 g/ml, by bioconversion with *G. oxydans* in batch mode. The yield decreases with increasing of initial glycerol concentration, furthermore highest yields was reached with fermentations of 20 and 50 g/l of glycerol

Experimental Procedures

Material, Culture Medium And Cultivation Conditions

The fermentationsof 25, 50 and 100 g/mL of glycerol wererealized according to the procedure previously describedby Acevedo et al.[9]. Changes of pH [9], glycerol concentrations and DHA production were registered during the period of glycerol fermentation.

Glycerol Consumption Determination

Glycerol consumption and DHA production were determined by gas chromatography, using anAgilent 4890D Gas Chromatograph. Calibration curve glycerol standard was conducted. Gas chromatograph for a Zebron ZBWax (30m x 0.53 mm ID x 0.5 um df) Phenomenex column was used, equipped with a flame ionization detector that operated at 300 ^{0} C. The temperature of the port of manual injection is 280 ^{0} C and the oven conditions were initial temperature of 190 ^{0} C with duration of 0.5 minutes and speed of 5 ^{0} C / min to a temperature of 260 ^{0} C and final time of 5 min.The run time of 19.5 min is used in the injection port of Split form in a ratio of 50 to 1, and a sample injection volume of 10 μL. Drag gas used is helium at a pressure of 5 psi, and the gases used in the flame detector were air - hydrogen relative 10-1 [10].

Results and Discussions

The Fig. 1 shows the glycerol concentration for fermentation of 20, 50 and 100 g/L of initial glycerol. The glycerol uptake by the bacteria was dependent with the initial concentration of this substrate which affects the fermentation time as shown in Fig. 1. Low initial concentration of glycerol requires lower process time indicating a slight inhibitory effect with substrate. The yield of glycerol fermentation decreases with increasing of initial concentration of glycerol, same production was observed at final time of fermentation for 50 and 100 g/L of glycerol as shown in Fig. 2. Rates of oxygen consumption and CO_2 evolution decreased by high concentrations of glycerol, the cell growth and all related metabolic activity are stopping and the energy spending just is destined for cell maintenance. The data of concentration of DHA got over the value of 25 g/L due to spontaneous evaporation in the samples of fermentation of 20 g/L glycerol. However, the glycerol oxidation was completedbefore 20 hours as shown in Fig. 1. The productions of DHA were very fast in the first 25 hours for the three fermentations, where exponential growth was observed and DHA was generated as primary metabolite which affected the growth of *G. oxydans* after 25 h, according with Sattler [11].

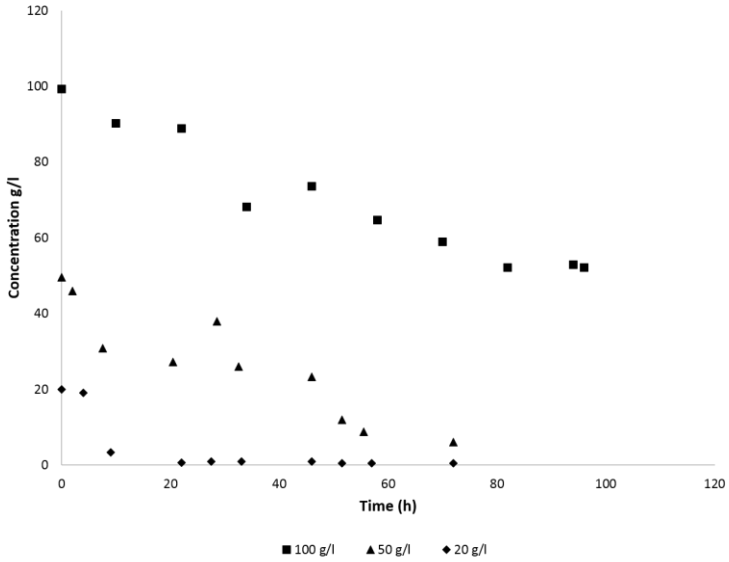

Figure 1: Glycerol concentration at fermentation of *Gluconobacter oxydans*

The consumption of glycerol is proportional with the production of dyhidroxyacetone, as it is observed in each showed value in Figs.1, 2 and 3. At 34 minutes, the data of glycerol concentration was out of the tendency line of the experiments with 100 g/l of initial glycerol, as shown in Fig. 3, maybe by the fermentation after sampling; this was suggested due to the decrease of glycerol concentration that was proportional with the increase DHA concentration in this point, as shown in Fig. 3.

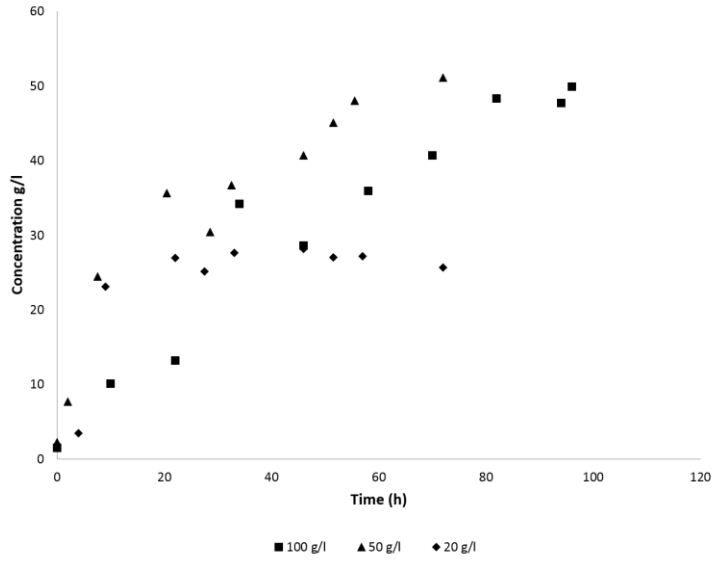

Figure 2: DHA concentration at fermentation of glycerol

In Fig. 2 for concentrations of glycerol 20 g /l, a concentration of DHA of around 30 g /l was found after 20 minutes, which was maintained constant. However, a different trend was observed with the fermentations of 50 and 100 g /l glycerol, where the concentration of product increased with exponential trend until 72 h and 96 h, respectively. The rate of consumption of glycerol decreased with high concentrations of glycerol due to the inhibition effect of substrate. After of 72 hours, concentrations of DHA close to 50 g/l were reached for those fermentations, value that is near to possible maximum concentration of DHA for the case of initial concentration of glycerol 50 g/l, this behavior can be due to inhibitory effect caused by growth rate when the initial substrate concentration increase from 50 to 100 g/l. Therefore, concentrations of DHA, around of 35 g/l, presented significant changes from 40 hours, where the rate of production was decreased, more rapidly in the fermentations of 100 g/L of glycerol, due to the catalyzed reaction of substrate-inhibitor-enzyme, affecting enzyme structure glycerol - dehydrogenase who no longer works efficiently, altering μmax, but not Ks [12].

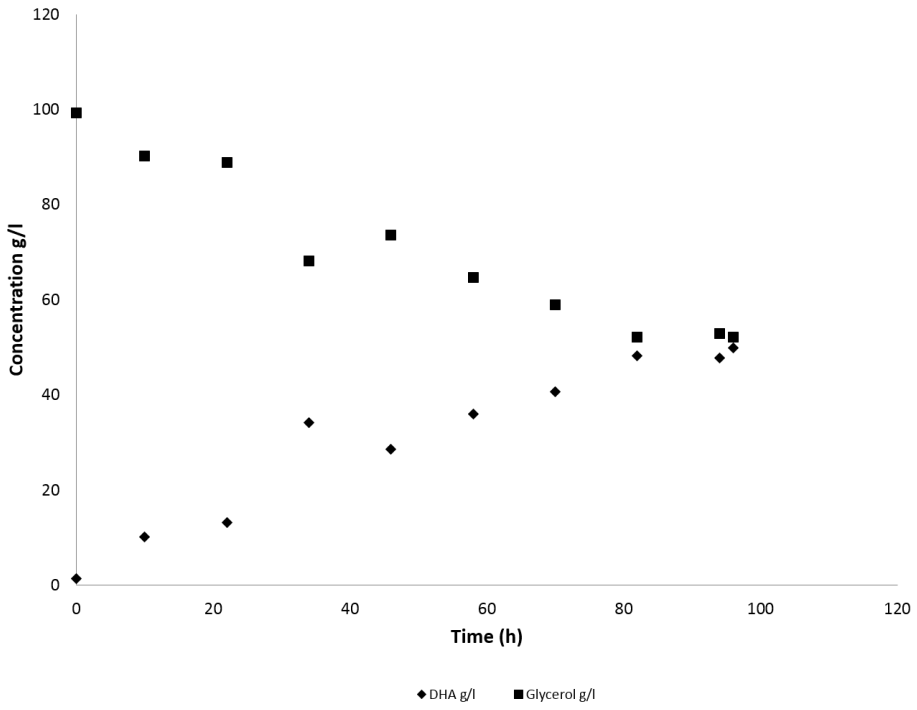

Figure 3: Evolution of concentrations of glycerol and DHA in the fermentation process of 100 g/l of glycerol

The yield of glycerol oxidation was calculated through the relation of values experimental and theoretical of DHA concentrations. In the Fig. 4 is observed as the yield decreased at high glycerol concentration corroborating the inhibition effect on cell behavior. As was mentioned above, the final concentrations of DHA at fermentation of 20 g/l of glycerol were higher than the theoretical value due to the

spontaneous evaporation of water, as shown in the Fig. 2. Thereby, the yield to this fermentation could be near to 99.99% as it can be verified with the final glycerol consumption in the Fig. 1, which is equal to zero with initial concentration of glycerol of 20 g/l. Moreover, the yield values reached in the fermentations of 50 and 100 g/l of glycerol were according with the final concentrations of DHA and glycerol, as shown in the Figs. 1, 2 and 3.Therefore, the spontaneous evaporation phenomenon of water was not observed at higher initial glycerol concentrations.

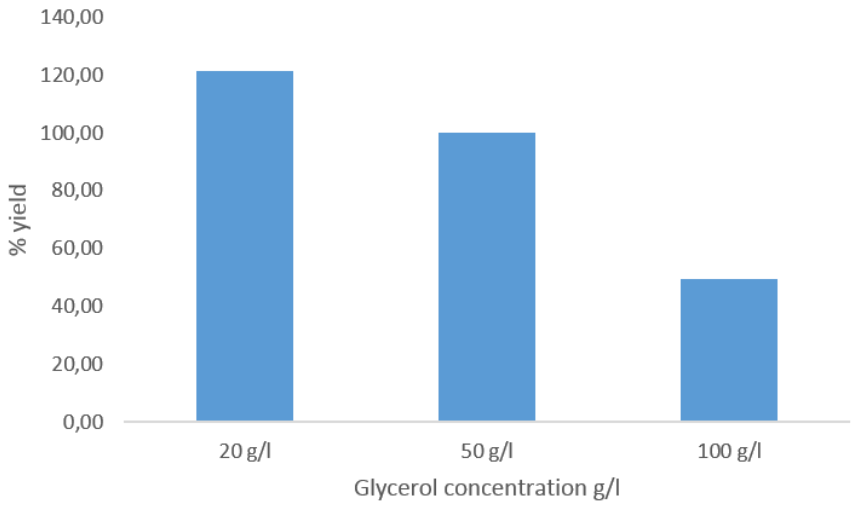

Figure 4: Yield of glycerol bioconversion with Gluconobacter oxydans

Conclusion

The DHA production is greater in the first 25 hours of fermentation without matter the initial glycerol concentration. However, the yield values decrease at higher substrate concentrations. Which was evidenced with the reached DHA concentrations that were similar in the bioconversion of 50 and 100 g/l of glycerol.

Spontaneous evaporation phenomenon of water was observed at glycerol fermentation with 20 g/l, which there was not presented with higher initial substrate concentrations.

The productions of DHA were very fast in the first 25 hours for the three fermentations, where exponential growth was observed and DHA was generated as primary metabolite which affected the growth of *G. oxydans* after 25 h, according with Sattler [11].

References

[1]. Rocha-Martin J, Acosta A, Berenguer J, Guisan J, Lopez-Gallego F, 2014, "Selective oxidation of glycerol to 1,3- dihydroxyacetone by covalently

immobilized glycerol dehydrogenases with higher stability and lower product inhibition", Bioresource Technology, 170, pp. 445-453.

[2]. Mishra R, Jain SR, Kumar A, 2008, "Microbial production of dihydroxyacetone", Biotechnol. Adv., 26 (4), pp. 293-303.

[3]. Enders D, Voith M, Lenzen A, 2005, "The dihydroxyacetoneunit - a versatile C3 building block in organic synthesis", Angew.Chem. Int. Edit., 44, pp. 1304-1325.

[4]. Pagliaro M, Ciriminna R, Kimura H, Rossi M, Pina CD, 2007, "From glycerol to value-added products", Glycerol Chemistry, Angew. Chem. Int. Ed., 46, pp. 4434-4440.

[5]. Hekmat D, Bauer R, Fricke J, 2003,"Optimization of the microbial synthesis ofdihydroxyacetone from glycerol with Gluconobacter oxydans", BioprocessBiosyst. Eng., 26, pp. 109-116.

[6]. Weiser JR, Zawaneh PN, Putnam D, 2011, "Poly(carbonate-ester)s of dihydroxyacetone and lactic acid as potential biomaterials", Biomacromolecules, 12 (4), pp. 977–986.

[7]. Zheng Z, Luo M, Yu JE, Wang JL, Ji JB, 2012, "Novel process for 1,3-dihydroxyacetone production from glycerol. 1. Technological feasibility study and process design", Ind. Eng. Chem. Res., 51, pp. 3715-3721.

[8]. Hekmat D, Bauer R, Neff V, 2007, "Optimization of the microbial synthesis of dihydroxyacetone in a semi-continuous repeated-fed-batch process by in situ immobilization of Gluconobacter oxydans", Process Biochemistry, 42, pp. 71–76.

[9]. Acevedo-Morantes M, De Ávila G, Realpe-Jiménez A, 2015, "Assessment of ph change in dihydroxyacetone production from glycerol", International Journal of Applied Engineering Research, 9 (24), pp. 29239-29246.

[10]. Wu J, Li M, Lin J, Wei D, 2011, "Determination of Dihydroxyacetone and Glycerol in Fermentation Process by GC after n-Methylimidazole Catalyzed Acetylation", Journal of Chromatographic Science, 49, pp. 375-378.

[11]. Sattler K, 1965, ''Studien zur Intensivierung der Umwandlung von Glyzerin in Dihydroxyaceton durch Acetobacter suboxydans", Zeitschr. Allg. Mikrobiol., 5, (2), pp. 136-146.

[12]. Mishra R, Rani J, Kumar A, 2008, "Microbial production of dihydroxyacetone", Biotechnology Advances, 26, pp. 293–303.