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Production Of Dihydroxyacetone By *Gluconobacter Oxydans* From Crude Glycerol

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The availability of petroleum sources in the near future is constrained, so nowadays renewable energy resources are largely focused. One of the most paramount renewable energy resources is the biodiesel. During the biodiesel engenderment, excess crude glycerol is engendered which contains methanol, free fatty acids and salt. The biodiesel engenderment results in 10% of glycerol as byproducts. The carbon content in the waste glycerol can be used to serve as a cheap carbon source for the production of high value chemicals, like dihydroxyacetone (DHA), succinic acid, ethanol, citric acid, propanediol, etc. *Gluconobacter oxydans* is a gram-negative rod or oval-shaped non-motile bacterium belonging to the family *Acetobacter*, capable of producing DHA. Under optimized condition of 10% (v/v) substrate concentration, 5% (v/v) inoculum at pH 6 and temperature 30°C, maximum yield of 81.64% (v/v) was obtained. Glyceric acid was produced as a byproduct during DHA production was confirmed by qualitative test, FTIR and GCMS analysis.

KEYWORDS

Crude glycerol, Dihydroxyacetone, Glyceric acid, *Gluconobacter oxydans,* GCMS, FTIR

1. INTRODUCTION

The necessity of swapping fossil fuels has boosted the search for renewable alternative energy sources and so the development of biofuels, such as biodiesel came into existence over the past two decades [1]. Biodiesels have been proposed as an alternative to petroleum based fuels as an energy source. Stoichiometrically, the biodiesel industry generates 10% crude glycerol (v/v) as a byproduct [2]. Crude glycerol superfluity has been created in the glycerol market, which must be utilized to augment biodiesel profitability [3,4]. Copious amount of impurities present in the crude glycerol, like methanol, soap, salt, etc., becomes major obstacles to find its application in industries [5]. The three hydroxyl functional group present in the glycerol offers the feasibility to produce high value added chemicals by catalytic or microbial conversion. Complete purification of crude glycerol is very expensive [6,7]. Hence, for the effective utilization of glycerol by microorganism either partial purification or separation of glycerol rich phase from crude glycerol becomes mandatory [8]. Moreover, the production of high value added compounds from crude glycerol will not only beneficiate its own market but also the biodiesel industry as its economic viability is closely linked to glycerol [9]. Therefore, there is a worldwide demand to efficiently use the potential of glycerol by converting it to various value added products, like 1,3 propanediol, butanol, citric acid, succinic acid and dihydroxyacetone [10,11,12].

Crude glycerol is a cheaper substrate and easily available with a great degree of reduction when compared with sugar. Conversion of glycerol to phosphoenolpyruvate or pyruvate generates twice the amount of reducing equivalents than does produce pyruvate from glucose or xylose [6]. Many microbes, such as *E.coli, Klebsiella, Enterobacter, Glucanobacter, Clostridium, Candida, Aspergillus* can convert crude glycerol into value added products by metabolizing glycerol

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aerobically or anaerobically as a sole carbon source, for the industrial fermentation process [13]. Glycerol has a wide application in different type of industries, like pharmaceutical, soaps, food, paint, cosmetics, toothpaste [14]. Apparently, the highest demand for glycerol is found in food and cosmetics industries, in which DHA or 1,3-dihydroxy-2-propanone stands to distinguish from other compounds obtained from glycerol. DHA has a wide application in cosmetic formulations as a self-tanning agent and also for the organic synthesis of very fine chemicals [15,16]. For the concern of economic, safety and quality requirement DHA can be produced from glycerol by *Glucanobacter oxydans* through a fermentation process.

2. MATERIAL AND METHOD

2.1 Crude glycerol

The left unattended crude glycerol sample was procured from the biodiesel unit in Bannari Sugars Pvt. Ltd., Tamil Nadu. Crude glycerol was highly viscous fluid with the initial glycerol concentration of 10.41% (v/v) and pH 9.6. The glycerol sample was partially purified using phosphoric acid and attained purity of 44.4% (v/v) as discussed [8,5]. This aids the microbes to utilize glycerol as an excellent viable carbon source for enhanced fermentative production of DHA.

2.2 Growth and maintenance of *Gluconobacter oxydans*

Gluconobacter oxydans strain (MTCC 904) was procured from microbial type culture collection, Chandigarh for the production of DHA. The lyophilized cells were revived in MRS (de Man, Rogosa and Sharpe) medium and placed on a rotary incubator shaker at 30°C, 150 rpm for 24 hr [17]. The revived cells were grown on agar slant and kept at 4°C and were subcultured every month. The pH of the medium was adjusted to 6.0 ± 0.2 with an addition of 1N NaOH/HCl. 15 g/L of agar was added in MRS medium to prepare the agar plates and slants [18].

2.3 Optimized and controlled fermentation

Fermentation experiments were carried out with five different initial substrate concentrations (40, 60, 80, 100 and 120 g/L) of crude glycerol and partially purified glycerol. For experiments, 5% v/v of inoculum from 24 hr grown seed culture medium was added to the partially purified glycerol as a substrate. For controlled fermentation, the flask was kept in an incubator shaker at 30°C and 150 rpm. Samples of fermentation mixtures were withdrawn every 4 hr for a total fermentation period of 52 hr. All the experiments were carried out in triplicate to access reproducibility and the average value in each experiment was reported. The specific growth rate and conversion rate was calculated.

2.4 Analytical methods and confirmatory test

The concentration of glycerol remains unutilized by microorganism was measured at regular interval at 480 nm [19]. The concentration *of G. oxydans* cell mass (or growth profile of the microbial cells) was monitored by measuring absorbance at 600 nm with a UV-Visible spectrophotometer and the dry cell weight was measured. The end product was confirmed by simple qualitative test, FTIR and GCMS analysis.

3. RESULT AND DISCUSSION

A hefty amount of impurities in crude glycerol influenced in inhibiting the growth of microorganism at all inoculum concentration. Stelmachowski [20] reported that growth rate (cell count) of gram negative *Gluconobacter oxydans* is much slower and lesser in crude glycerol in all inoculum concentration when compared with partially purified glycerol with phosphoric acid. At pH 6 and temperature 30°C, the partially purified glycerol of 10% (v/v) substrate concentration with 5% inoculum shows significant biomass production and product formation when compared other substrate concentration. DCW, substrate utilization and product formation of *G. oxydans* (10% (v/v)) glycerol concentration were represented in figure 1. At the end of the fermentation process, results reveal that 4 and 6% (v/v) glycerol sources were rapidly exhausted by *G. oxydans* when compared with other substrate concentration. Glycerol concentration greater than 10% (v/v), that is 12% (v/v) was

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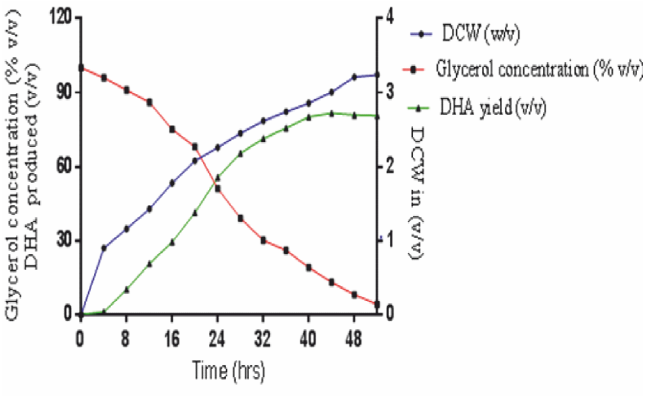


Figure 1. *DHA production at pH 6 and temperature 30°C on 10% (v/v) PPG concentration*

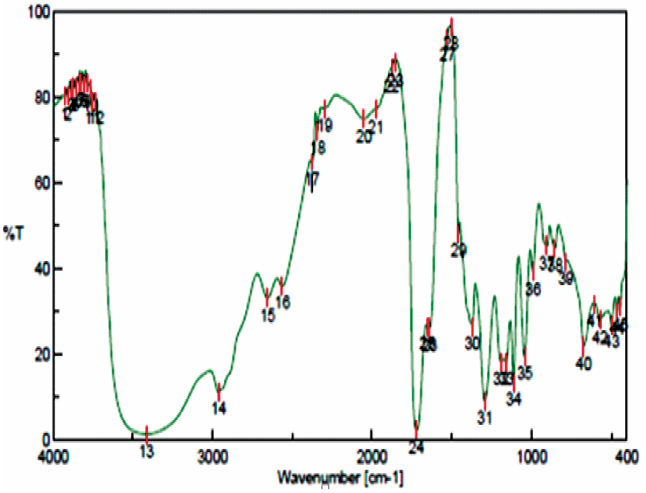


Figure 2. *FTIR analysis of DHA*

Table 1. *FTIR data interpretation of wave number to the bond produced by Gluconobacter oxydans*

Wave number/cm	Assignment	Sample
1721.16	C=O, stretch, strong, ketone	DHA
3730.62 and 3753.76	O-H, stretch, strong, alcohol	
2964.05	C-H, stretch, strong, alkane	
1721.16	C=O, stretch, strong, acid	Glyceric acid
3417.24	O-H, stretch, strong, acid	
1191.79	C-O, stretch, strong, acid	
3730.62 and 3753.76	O-H, stretch, strong, alcohol	

lingeringly unutilized and takes much longer lag phase when compared to 10% (v/v) substrate concentration. It was reported that at higher concentration the metabolic action is decreased, which ultimately results in slower growth of both organisms [17]. At an optimized condition of pH 6, temperature 30°C with 10% substrate concentration and 5% inoculum concentration, *Gluconobacter oxydans* yields 81.64% (v/v) of DHA.

3.1 Confirmatory test

The resultant product obtained after fermentation was centrifuged and further analyzed qualitatively. The Seliwanoff's reagent consists of resorcinol and concentrated hydrochloric acid. Keto group undergoes dehydration to yield furfural derivative which rapidly forms complex cherry red colour on heating indicates the presence of keto group in the given sample. DHA also contains the keto group in it. Glyceric acid was also obtained as a byproduct during the production of DHA which was reported by previous literature was confirmed by qualitative [21] and FTIR analysis. The FTIR Spectrum for the sample produced by *Gluconobacter oxydans* was recorded using Perkin Elmer spectrum RXI FTIR spectrometer by using potassium bromide in the transmittance range of 400-4000/cm. This is a highly sensitive and quick method to achieve high quality spectrum with a signal to noise ratio when compared with other dispersive instruments. The structural assignment of the samples shows the presence of dihydroxyacetone and glyceric acid from figure 2 has been correlated with the following frequencies as shown in table 1.

The compounds detected in GCMS of *Gluconobacter oxydans* confirms the presence of DHA and glyceric acid, which was evident from the MS data recorded against the sample injected with respect to a retention time of about 8.761, 13.64 and 14.033. 2,3-hydroxypropanoic acid is also commercially known as glyceric acid. Many compounds possess dimethyl silyl because of the derivatization technique of silylation using TMSI. Silylation produces silyl derivatives which are more volatile, less stable and more thermally stable. The results are depicted in figure 3.

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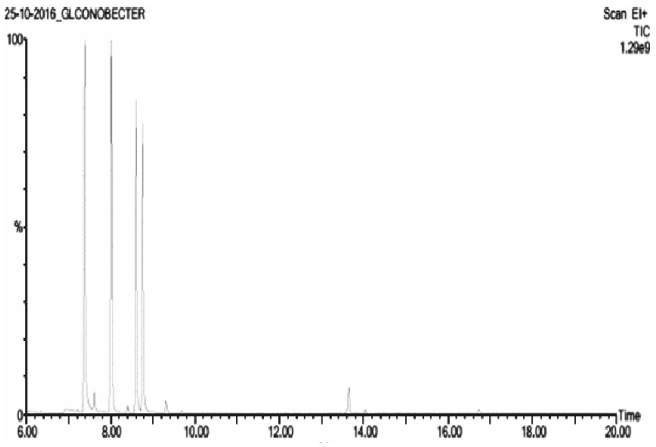


Figure 3. *GCMS analysis of DHA*

4. CONCLUSION

Biodiesel is labelled to be a future renewable alternative fuel, which produces crude glycerol as a waste byproduct. The bioconversion crude glycerol to value added products is a sustainable approach compared to chemical conversion. There are many products which can be made from crude glycerol which can be used in cosmetics, pharmaceuticals, feed products, etc. Bioconversion approaches seem to a viable source for economic development and also towards the maintenance of nature. Dihydroxyacetone is a better choice to valorize the excess glycerol obtained as a byproduct in biodiesel production. *Gluconobacter oxydans* is capable of utilizing glycerol as a sole carbon source through a biological fermentation process. However, the impurities present in crude glycerol inhibit or slow down the growth of organisms, hence partial purification or separation of glycerol rich is mandatory.

REFERENCES

1. Katryniok, B. 2011. Selective catalytic oxidation of glycerol : Perspectives for high value chemicals. *Green Chem.,* 13 (8):1960-1979.
2. Rahmat, N., A.Z. Abdullah and A.R. Mohamed. 2010. Recent progress on innovative and potential technologies for glycerol transformation into fuel additives: A critical review. *Renew. Sust. Energy Rev.,* 14 (3):987-1000.
3. Ardi, M.S., M.K. Aroua and N.A. Hashim. 2015. Progress, prospect and challenges in glycerol purification process : A review. *Renew. Sustain. Energy Rev.,* 42:1164-1173.
4. Quispe, C.A.G., C.J.R. Coronado and J.A. Carvalho. 2013. Glycerol : Production, consumption, prices, characterization and new trends in combustion. *Renew. Sustain. Energy Rev.,* 27:475-493.
5. Chozhavendhan, S., *et al*. 2017. Production of ethanol by *Zymomonasmobilis* using partially purified glycerol. *J. Energy and Env. Sustainability.* 4:15-19.
6. Garlapati, V.K., U. Shankar and A. Budhiraja. 2016. Bioconversion technologies of crude glycerol to value added industrial products. *Biotech. Rep.,* 9:9-14.
7. Zheng, X., *et al*., 2016. Effects of oxygen transfer coefficient on dihydroxyacetone production from crude glycerol. *Braz. J. Microbiol.,* 47(1):129-135.
8. Chozhavendhan, S., *et al*. 2016. Purification and characterization of waste stream glycerol derived from biodiesel industry. *J. Env. Biology.* 37:1529-1534.
9. Kenar, J.A. 2007. Glycerol as a platform chemical : Sweet opportunities on the horizon? *Lipid Tech.,* 19:249-253.
10. Poljungreed, I. and B. Boonyarattanakalin. 2017. Dihydroxyacetone production by *Gluconobacter frateurii* in a minimum medium using fed–batch fermentation. *J. Chem. Tech. Biotech.,* 92(10):2635–2641.
11. Sadhukhan, S., R. Villa and U. Sarkar. 2016. Microbial production of succinic acid using crude and purified glycerol from a *Crotalaria juncea* based biorefinery. *Biotech. Report.* 10 : 84-93.
12. Vivek, N., A. Pandey and P. Binod. 2016. Biological valorization of pure and crude glycerol into 1,3-propanediol using a novel isolate *Lactobacillus brevis* NIE 9.3.3. *Bioresour. Tech.,* 213:222–230.
13. Solomos, B., *et al*. 1995. Comparison of the energetic efficiencies of hydrogen and oxochemicals formation in *Klebsiellapneumonia* and *Clostridium butylicum* during anaerobic growth on glycerol. *J. Biotech.,*

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39:107-117.
14. Colin, T., *et al*. 2001. Effects of acetate and butyrate during glycerol fermentation by *Clostridium butyricum*. *Curr. Microbiol.,* 43 (4):238-243.
15. Bauer, R., *et al.* 2005. Study of the inhibitory effect of the product dihydroxyacetone on *Gluconobacter oxydans* in a semi-continuous two-stage repeated-fed-batch process. *Bioprocess Biosyst. Eng.,* 28 (1):37-43.
16. Hekmat, D., R. Bauer and J. Fricke. 2003. Optimization of the microbial synthesis of dihydroxyacetone from glycerol with *Gluconobacter oxydans*. *Bioprocess Biosyst. Eng.,* 26(2):109-116.
17. Lidia, S., *et al.* 2014. Effect of glycerol and dihydroxyacetone concentrations in the culture medium on the growth of acetic acid bacteria *Gluconobacter oxydans. ATCC 621 Eur. Food Res. Tech.,* 239 (3):453-461.
18. Dikshit, P.K. and V.S. Moholkar. 2016. Optimization of 1,3–dihydroxyacetone production from crude glycerol by immobilized *Gluconobacter oxydans* MTCC 904. *Bioresour. Tech.,* 216:1058-1065.
19. Paolo, B. and D.B. Laura. 2005. An alternative spectrophotometric method for the determination of free glycerol in biodiesel. *Eur. J. Lipid Sci. Tech.*, 107(3):153-157.
20. Stelmachowski, M. 2011. Utilization of glycerol, a byproduct of the transesterification process of vegetable oils : Arc view. *Ecological Chemistry and Eng.*, 18 (1):9-30.
21. Chozhavendhan, S., *et al.* 2014. Bioconversion of crude glycerol into glyceric acid : A value added product. *Int. J. Chem. Tech. Res.,* 6(12):5053-5057.

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