Biocatalytic transformation of bioglycerol to diols

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Abstract: Current research has been aimed at conversion of biobased platform molecules into fuels and multiple commodity products. In our recent study it was found that bio-glycerol, a co-product of biodiesel industry can be efficiently converted to useful products such as 1,3-propanediol (1,3-PDO) as well as to C_4 diol *viz.* 2,3-butanediol (2,3-BDO) by the members of the Enterobacteriaceae family. Using cell free supernatant almost complete conversion of bioglycerol was obtained yielding 17 g/L 2,3-BDO, which is a raw material in the rubber industry. The use of extracellular enzyme as a biocatalyst will facilitate the cost effective production by way of efficient utilization of the substrate and highest selectivity to the desired product.

Keywords: Bioglycerol, Biocatalysis, 2,3-butanediol.

1. Introduction

Implementation of bio refineries is an upcoming alternative to overcome both the fossil fuel dependence and the limited profitability of the existing technologies[1]. In this regard, topical biodiesel is of interest which is obtained by transesterification reaction of renewable sources in which glycerol is a valuable by-product[2]. This bioglycerol is about 10% of total biodiesel production creating the formidable problem of its disposal. Conversion of such bioglycerol to value added products by microbial route is of greater importance in promoting biodiesel industrialisation on a larger scale. Bioconversions have distinct advantage of biodegradability with respect to bio catalysts, substrates, intermediates, and the resulting products and by-products and invariably such processes utilize water as a solvent[3].

Bacterial strains were screened and four of the strains belonging to the Enterobacteriaceae family were found to efficiently convert bio-glycerol to useful products such as 1,3-PDO, 2,3-BDO (major), ethanol and acetoin, with the highest conversion of 69% and 79%[4]. However, the conversion was carried out containing whole cells, interestingly we simultaneously studied the conversion of bioglycerol with the cell free supernatant which proves to be promising with regards to industrialisation.

2. Experimental

Bacterial strains were obtained from NCIM, bioresource centre, NCL, Pune, India. Stock cultures of selected strains were maintained on nutrient agar (Difco) slants. The culture was transferred every 2 weeks and incubated for 24 h at 30ºC.

Assay: Culture was inoculated in 10 mL of nutrient broth containing 1% glycerol incubated in orbital shaker at 30ºC, 150rpm in a thermostated room (26ºC). 24 h to 48 h grown cultures was centrifuged at 10,000 rpm for 10min at 4ºC. The supernatant liquid was used to carry out the transformation of bioglycerol. Cell free supernatant, 4mL was suspended in 6mL of (30g/L) bioglycerol. The reaction tubes were placed in orbital shaker at 30ºC, 150rpm in a thermostated room (26ºC). Control was also maintained. Experiments were performed in replicates. 1.5ml of samples withdrawn was centrifuged at 1,000 rpm for 10min at 4 ºC to separate the cells. Clear supernatant was used for the determination of products (ethanol, acetoin, 2,3-BD, and 1,3-PDO) by gas chromatography.

3. Results and discussion

As can be seen from Table 1, highest conversion of (99%) bioglycerol was obtained by *Klebsiella pneumoniae* 5215, using cell free supernatant to give 15 g/L 2,3-BDO and other products at less than 8 g/L. However, high yields of 17 g/L 2,3-BDO, 10 g/L acetoin, 2-3 g/L 1,3-PDO and ethanol were obtained by using *Enterobacter aerogenes* 2695, converting 98% bioglycerol. *Enterobacter cloacae* 2562 also showed similar results with 16 g/L 2,3-BDO. Initially from 24h, conversion was found to increase consistently and almost complete conversion was obtained by 96h. Bioglycerol was used as the only source of carbon and as a nutrient for the microorganism to carry out the conversion into value added products. There is no literature reported till date considering cell free supernatant and only bioglycerol for direct conversion of bioglycerol into 2,3-BDO and 1,3-PDO. Extracellular produced enzyme (biocatalyst) plays a direct role in carrying out the transformation and its further identification studies are in progress. Based on these results the bioglycerol transformation to various products is shown in Figure 1.

Sr. No.	Bacterial strains (NCIM)	Conversion (%)	Time (h)	Products (g/L)			
				2,3-BDO	1,3-PDO	Acetoin	Ethanol
1	*Enterobacter aerogenes* 2695	98	96	17	1	8	3
2	*Klebsiella pneumoniae* 5215	99	96	15	2	10	3
3	*Enterobacter aerogenes* 2694	70	96	12	4	-	3
4	*Enterobacter cloacae* 2562	80	96	16	-	5	2

Table 1. Biocatalytic conversion using different bacterial strains at the end of 96h.

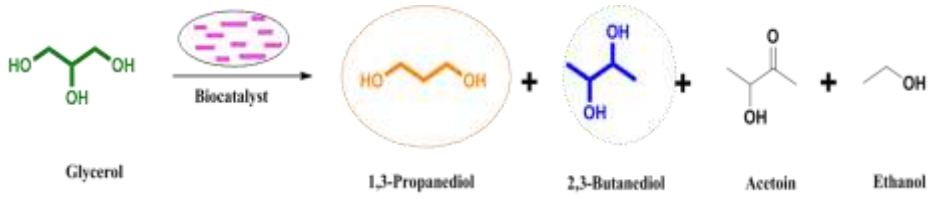

Figure 1. Reaction scheme for bioglycerol conversion to value added products.

4. Conclusions

Utilization of biobased materials for sustainable society is of much interest. Biodiesel derived glycerol is one such example which can be catalytically converted into exploitable significant products such as 2,3-BDO and 1,3-PDO. These products have high demand in polymer and medical industry. Cell free supernatant – biocatalyst could bring out high conversion of glycerol to 2,3-BDO which is the major highlight of the study. An efficient and a greener route by means of biocatalysis will eventually add on to the process for sustainability in future and further explanation is in progress in our laboratory.

References

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