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A novel biological route for 1, 3-propanediol synthesis through transesterification of cottonseed oil

Mandar Karve, Jay J.Patel, V.K.Sinha, Nirmal K.Patel*

Department of Industrial Chemistry, Institute of Science & Technology for Advanced Studies and Research, Vallabh Vidyanagar-388120, Gujarat, (INDIA)

E-mail : drnirmalpatel@yahoo.com

ABSTRACT

Transesterification of cottonseed oil was carried out using methanol to produce crude glycerol. Glycerol shall be converted to 1,3-propanediol (1,3-PDO) by chemical as well as biological route. In chemical synthesis of 1,3-PDO high energy and expensive catalysts like Cr, Ir and Ag are required which leads to high costs of 1,3-PDO production. The bioconversion route for 1,3-PDO synthesis is a attractive alternative to the traditional chemical synthesis. Enterobacter aerogen (E. aerogen) was utilized for bioconversion of crude glycerol. The bioconversion was carried out at 25^0C, 30^0C, 35^0C and 40^0C temperature and atmospheric pressure for 2, 4, 6, 8, 10, 12days using tryptone soya broth medium. Best result was obtained at 30^0C where 0.615mole of 1,3-PDO produced per mole of glycerol. The 1,3-PDO was analyzed by gas chromatography and FTIR.

KEYWORDS

Bioconversion;
Enterobacter aerogen;
Transesterification;
Crude glycerol;
1,3-propanediol.

INTRODUCTION

A major by-product of biodiesel industry is glycerol which is commonly known as glycerin[1]. Glycerol is a trihydric alcohol, miscible with water, ethyl acetate and dioxane while immiscible with chloroform, benzene and ether. It is a colorless, odorless, viscous and hygroscopic liquid with a high boiling point. Pure glycerol is a versatile product and readily compatible with other substances[2,3].

Glycerol founds a broad diversity of application as it is used in various industries such as paints, automotive, textile, pharmaceuticals, cosmetic, food, paper industries due to the unique combination of physical and chemical properties[4]. Glycerol can also be utilized as a renewable source for biodegradable products and also find applications in green refinery process[5].

The glycerol is produced as a by-product in fat processing, ethanolic fermentation of glucose and biodiesel industry in a constantly increasing amount. Among which biodiesel industry have glut of crude glycerol as by-product which results in a serious environmental and disposal problems. Approximately 12.2 million metric ton biodiesel is produced which generates 1.22 million metric ton crude glycerol. The massive glycerol production also forces a collapse in its market price[6]. On the other side, demand of petrol and diesel as a fuel in world is increasing day by day while the petroleum resources are decreases continuously. Fuel crisis has been affected the worldwide

economy[7]. In the present scenario, biodiesel which is obtained from 100% renewable resources provides an alternative fuel option for future. The biodiesel is a very important product for now and a future aspect and use of it helpful in protection of environment. The crude glycerol from biodiesel process can be utilized for further synthesis or application then biodiesel may available in economic price[8-10].

Glycerol can be converted to variety of products such as 1,3-propanediol (1,3-PDO), 1,2-propanediol, succinic acid, ethanol, butanol using chemical and biological method. As specially when desired product is 1,3-PDO, it can be produced chemically by hydration of acrolein[11-14]. But it consists high energy consumption, toxic intermediates and expensive catalysts like Ag, Ir and Cr are required which leads to high costs of 1,3-PDO production[15,16]. The biological route was carried out at or slightly above the room temperature and atmospheric pressure. Bioconversion of crude glycerol into 1,3-PDO can be carried out using microorganisms like Pseudomonas, Escherichia coli, Clostridiumbutyricum and Citrobacterfreundii[17-20].

Bioconversion of crude glycerol provides substrates for the production of biodegradable polymers which directly benefit to the environment. An interesting example is a polytrimethylene terephthalate (PTT) production in which 1,3-PDO is used as monomer. PTT has unique physiochemical properties in the fiber industry and other applications in cosmetics, foods, lubricants and medicines. Also 1, 3-PDO can be formulated into laminates, composites, adhesives, powder coating and as an anti-freeze agent. It can be used in manufacturing of polyester, polyurethane and polyol[21,22].

There so, in the present work transesterification of cottonseed oil was carried out with methanol to obtained crude glycerol. The crude glycerol was converted into 1,3-PDO using E. aerogen strain in tryptone soya broth medium at 25^0C, 30^0C, 35^0C and 40^0C temperature for 2, 4, 6, 8, 10, 12days. 1, 3-PDO was characterized by FTIR and gas chromatography (G.C.).

MATERIALS AND METHODS

Materials

Cottonseed oil, sodium hydroxide and nutrient broth medium, tryptone soya broth medium were purchased from Sigma Aldrich while solvents viz. chloroform and methanol were purchased from Merck India Private Ltd. Any other solvent were used of A.R grade.

Methods

Transesterification of cottonseed oil

Transesterification of cottonseed oil is carried out by methanol and sodium hydroxide (1% based on cottonseed oil) at 65^0C for 2.5hrs. During the process methyl ester and crude glycerol was produced with 3:1 ratio. The reaction mass was cooled and transfer into separating funnel where it was allowed for layer separation. Two layers viz. lower layer rich in glycerol and upper layer rich in methyl ester are formed for 1hr. Lower layer is separated and neutralized by concentrated HCl to obtain crude glycerol while methyl ester is used for biodiesel application.

Bioconversion of crude glycerol to 1, 3-propanediol

E. aerogen strain was inoculated in previously autoclaved nutrient broth medium (composition shown in TABLE: 1) for 24hrs. The prepared pre-culture medium was then utilized for bioconversion of crude glycerol. The tryptone soya broth medium (30gm) (composition shown in TABLE: 2) and crude (50gm) glycerol was dissolved in 1liter distilled water. The prepared medium was sterilized in an autoclave at 121^0C at 15lb/in^2 for 30minutes. Pre-culture medium was added in each 250ml flasks containing 150ml medium. The flask was incubated at 200rpm at 25^0C, 30^0C, 35^0C and 40^0C for 2, 4, 6, 8, 10, 12days.

TABLE 1 : Nutrient broth medium composition

Sr. No.	Composition	Gms/liter
1.	Peptone	10
2.	Beef extract	10
3.	Sodium chloride	05

TABLE 2 : Tryptone soya broth medium composition

Sr. No.	Composition	Gms/liter
1.	Pancreatic digest of casein	17.0
2.	Papaic digest of soyabean meal	3.0
3.	Sodium chloride	5.0
4.	Dipotassium hydrogen phosphate	2.50
5.	Dextrose (glucose)	2.50

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TABLE 3 : 1,3-PDO yield at different temperature

Sr. No.	Days	Yield(mol/mol)			
		25^0C	30^0C	35^0C	40^0C
1.	2	0.062	0.295	0.153	0.086
2.	4	0.112	0.310	0.205	0.133
3.	6	0.198	0.483	0.285	0.156
4.	8	0.278	0.580	0.362	0.202
5.	10	0.361	0.614	0.412	0.220
6.	12	0.361	0.614	0.412	0.221

Separation

The biomass was separated by centrifugation at 3000rpm. Then 1,3-PDO was isolated by chloroform as solvent in extraction process. The chloroform was recovered by distillation.

Gas chromatography

The resulting 1,3-PDO was characterized by Gas chromatograph (GC) using Perkin Elmer auto system

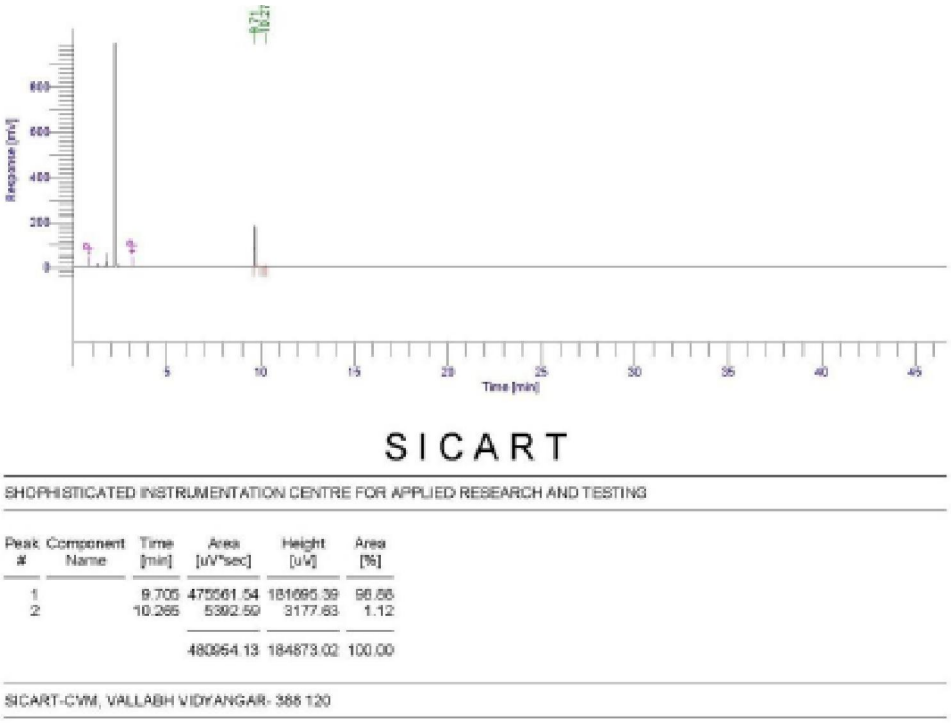

Figure 1

XL instrument using PE-FFAP column.

FTIR spectroscopy

The 1,3-PDO was characterised by FTIR spectroscopy using Perkin Elmer spectrum GX instrument.

RESULT AND DISCUSSION

The crude glycerol obtained during transesterification of cottonseed oil, contains impurities such as unreacted methanol and fatty acids. Generally excess methanol was used to drive the transesterification and if methanol was not recovered properly then it may present in glycerol layer in trace amount along with fatty acid. The crude glycerol contains unreacted methanol and sodium hydroxide in trace amount, do not affect the bioconversion process even high pH level of it help the process. So the cost of bioconversion process was decreased as crude glycerol was used directly without refining process.

Bioconversion was carried out with 50gm/L glycerol concentration at 25^0C, 30^0C, 35^0C and 40^0C for 2, 4, 6, 8, 10, 12days using E. aerogen strain. Among various temperatures, at 30^0C, highest 1,3-PDO yield was obtained as shown in TABLE 3. Optimum yield was obtained during 10days of bioconversion process, while almost same yield was noted in further days. Glycerol conversion was found maximum at 30^0C temperature while at 35^0C temperature lower yield obtained. As the bioconversion temperature was raise to 40^0C, lowest yield was obtained. As the bioconversion process time increases yield of 1,3-PDO was increases at certain. After 10days of bioconversion process, constant yield

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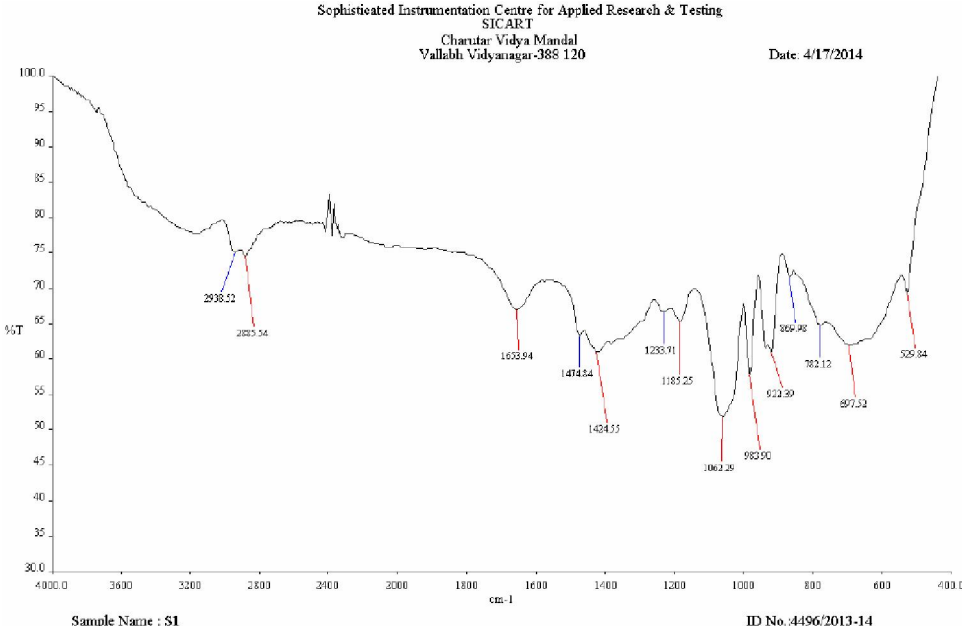

Figure 2

was observed.

So it was concluded that during 10days process at 30^0C, crude glycerol gives best results which were tabulated in TABLE 3.

Gas chromatography

The chromatograph is shown in Figure 1. The bioconversion product and their quality were determined using Gas Chromatograph using a PE-FFAP column. The sample was run into the GC instrument. At 9.706min, 1,3-PDO was observed which was 98.88% pure. While at 10.265min impurity was observed present in sample.

FTIR spectroscopy

The FTIR spectrum of 1,3-PDO is shown in Figure 2. The structure of the 1, 3-PDO contains primary alcohol and alkane substitution. The strong band observed between 2938.52 – 2885.54cm^{-1}.due to C—H streching frequency. The medium vibration observed at 1653.94cm^{-1} which confirms the present of C—C—C streching. The —CH_2— bending observed at 1474.84cm^{-1}. Due to primary alcohol strong O—H bending vibration and strong C—O streching vibration observed at 1062.29cm^{-1} and 1233.71cm^{-1}. So above FTIR spectrum confirms the 1,3-PDO

CONCLUSION

Crude glycerol obtained during the transesterification of cottonseed oil, was converted to the 1,3-PDO using E. aerogen strain. Best results were obtained in 10days process with at 30^0C temperature. This 1,3-PDO can be utilized in the manufacturing of polytrimethylene terephthalate (PTT) which is a biodegradable polymer.

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