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Novel Method of Bioconversion of Waste Glycerol Acquired during Biodiesel Production

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Abstract

The bioconversion of glycerol was carried out using Lactobacillus strain. Lactobacillus was grown on nutrient broth. The pre-culture was added to MRS (De Man, Rogosa and Sharpe) broth medium where glycerol was used as carbon source to acquire 1,3-propanediol (1,3-PDO). Bioconversion of glycerol was carried out at 20°C, 25°C, 30°C, 35°C and 40°C, using Lactobacillus strain. The atmospheric pressure and temperature were maintained for 1, 2, 3 and 4days. Optimum result was obtained at 35°C and 3rd day where 0.59mole of 1,3-PDO produced per mole of glycerol. Resulted 1,3-PDO was characterized by Gas chromatography (GC), Fourier Transform Infrared Spectroscopy (FTIR) and Proton Nuclear Magnetic Resonance (1H NMR) techniques.

Keywords: ***Waste glycerol; Biodiesel; Bioconversion; 1,3-propanediol; Lactobacillus strain***

Introduction

The Bioconversion is also known as biotransformation. In this live microorganisms used to carry out reaction. If this reaction carried out non-biologically, process would be unfeasible or costlier. Bioprocessing combines the disciplines of microbiology, chemical engineering and biochemistry [1,2]. The microorganisms convert a substance into a chemically modified form. For example, the bioconversion of Progesterone to 11-alpha-Hydroxyprogesterone by *Rhizopus nigricans*, bioconversion of organic materials such as plant or animal waste into usable products or energy sources using various microorganisms [3].

The bioconversion is generally carried out by three different methods such as enzymatic hydrolysis: which consist single source of feed stock mixed with enzymes, synthesis gas fermentation: which consist blend of feedstock is gasified and third method is C.O.R.S. (Continuous oxidation/ reduction system) and Grub Composting: where microorganisms are feed on feed stocks which reduce and convert in to high quality product [4].

The bioconversion of various feedstocks are carried out such as materials derived from plant or animal waste, paper, tires, fabric, construction materials, municipal solid waste, sludge, sewage, agricultural residues such as sugarcane bagasse, corn,

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cellulose and crude glycerol from biodiesel industry into useful chemicals viz. carboxylic acids: acetic acid, propionic acid, butyric acid, ketones: acetone, methyl ethyl ketone, diethyl ketone and alcohols: 1,3-PDO, ethanol, propanol, n-butanol, secondary alcohols: isopropanol, 2-butanol, 3-pentanol using various microorganisms [5,6].

Bioconversion of waste glycerol, sugarcane biomass, xylose, hexose etc. is carried out at room temperature and at atmospheric pressure using various microorganisms such as *Pseudomonas, Enterobacter aerogen, Escherichia coli, Lactobacillus, Citrobacter freundli, Klebsiella pneumoniae, Clostridium pasteurianum, Enterobacter agglomerans, Bacteroides ruminicola, Thermoanaerobacterium, Thermosaccharolyticum, Gluconobacter oxydans, Anaerobiospirillum succiniciproducens, Propionibacterium acidipropionici, Propionibacterium and Clostridium propionicum* [7-12].

Falling into the above trends, in the present work bioconversion of crude glycerol is carried out using *Lactobacillus* strain. Glycerol was obtained as by-product from the biodiesel industry. For every 2240 lit of biodiesel produced, 224liter of glycerol was released. Glycerol also can be converted to 1,3-PDO by chemical method but process is not cost effective [13]. The crude glycerol was directly used for the bioconversion method which resulted into 1,3-PDO using Lactobacillus strain. Lactobacillus is a heterofermentative bacterium, known to inhabit the gastrointestinal tracts of humans, pigs, birds and other animals. The main advantages of using this microorganism are its ability to produce 1,3-PDO, and the fact that it is not pathogenic or genetically modified [14].

1,3-PDO was the oldest known fermentation product and was identified in 1881 by August Freund, as a product of glycerol fermentation by *Clostridium pasterianum*. It is also known as a trimethylene glycol, 1.3-dihydroxypropane, propane-1,3-diol. Molecular formula of the 1,3-PDO is C3H8O2, molecular mass 76.09gm/mole, the boiling point is 210 - 212˚C and melting point is -280˚C. 1,3-PDO is a typical product of glycerol fermentation. It is a valuable chemical intermediate potentially used in the manufacture of polymers i.e. polyesters, polyethers, polyurethanes etc., cosmetics, lubricants, medicines, and as an intermediate in the synthesis of heterocyclic compounds [15,16].

1,3-PDO can be used for the production of polyester polyol and polyester resin which straight advantage to the environment. In the polyester polyol, 1,3-PDO is used as monomer. Polyester polyol is used in coating industry as well for adhesion properties [17].

There so, in the present work the glycerol was converted into 1,3-PDO using *Lactobacillus* strain in MRS broth medium at 20˚C, 25˚C, 30˚C, 35˚C and 40˚C temperature for 1, 2, 3, 4 days. 1, 3-PDO was characterized by GC, FTIR and 1H NMR spectroscopy.

Materials and Methods

Materials

Sodium hydroxide, nutrient broth medium and MRS broth medium were purchased from Sigma Aldrich. Chloroform was purchased from Merck India Private Ltd., Mumbai. Other solvent and chemicals were of A.R grade and used after routine purification.

Methods

Bioconversion of glycerol

Nutrient broth medium (composition shown in TABLE 1) was autoclave and then *Lactobacillus* strain was inoculated in it for 24hrs. The prepared pre-culture medium was then utilized for bioconversion of glycerol.

The MRS broth medium (composition shown in TABLE 2) was dissolved in 1liter distilled water where 60 gm glycerol was also added. The prepared medium was sterilized in an autoclave at 121˚C at 15 lb/in 2 for 30 min. 250 ml Erlenmeyer flasks containing 150 ml of MRS broth medium and glycerol was added with pre-culture nutrient medium. The flask was incubated at 500rpm at 20˚C, 25˚C, 30˚C, 35˚C and 40˚C and at atmospheric pressure for 1, 2, 3, 4 days.

TABLE 1. **Nutrient broth medium composition.**

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

TABLE 2. **Lactobacillus MRS broth composition.**

Sr. No.	Composition	Gms/liter
1.	Proteose peptone	10.00
2.	Beef extract	10.00
3.	Yeast extract	5.00
4.	Dextrose	20.00
5.	Polysorbate 80	1.00
6.	Ammonium citrate	2.00

Separation

The biomass was separated using centrifugation at 3000rpm. Then 1,3-PDO was separated using solvent extraction method using chloroform as solvent.

Purification

Mixture from extraction process contains high amount of 1,3-PDO, chloroform and less volume of glycerol and water was distilled in vacuum distillation where chloroform and water was removed. The 1,3-PDO was further purified by distillation.

Gas chromatography

1,3-PDO was characterized by Gas chromatograph (GC) using Perkin Elmer auto system XL instrument using PE-FFAP column. The sample was run into GC for 45min where nitrogen was used as carrier gas.

FTIR spectroscopy

The 1,3-PDO was characterized by FTIR spectroscopy using Perkin Elmer spectrum GX instrument by Nujol mull method.

1HNMR spectroscopy

1,3-PDO was characterized by 1H NMR spectroscopy using Bruker Advance III 400MHZ instrument. Chemical shifts (δ) were reported in ppm, using standard solvent as internal standard.

Result and Discussion

The crude glycerol obtained from biodiesel production was used for the bioconversion process. The crude glycerol contains impurities such as sodium hydroxide, unreacted methanol and fatty acids in bit amount which do not affect the bioconversion process. It helps the process as crude contains high basic pH level. So the cost of bioconversion process was decreased as crude glycerol was used straight without purifying.

Optimization of Reaction Parameter

- **Effect of temperature**

The effect of temperature on the synthesis of 1,3-PDO was optimized and the results were tabulated in TABLE 3. Bioconversion was carried out with 60gm/L glycerol concentration at 20˚C, 25˚C, 30˚C, 35˚C and 40˚C using *Lactobacillus* strain. Among various temperatures, at 350C, highest 1,3-PDO yield was obtained as shown in TABLE. 3. Optimum yield was obtained during 3days of bioconversion process, while almost same yield was noted in fourth day. Initially at 20˚C temperature, lowest yield was observed and further the 1,3-PDO yield was increased till 35˚C temperature while beyond 350C temperature yield was decreased. As Lactobacillus strain was not more survive and grow at 40˚C temperature, lower yield was result and beyond 40˚C, bioconversion of glycerol to 1,3-PDO was not take place. So optimum yield was observed at 35˚C temperature for bioconversion of glycerol.

- **Effect of reaction time**

The effect of reaction time on the synthesis of 1,3-PDO was optimized and the results were tabulated in TABLE 3. Bioconversion was carried out with 60 gm/L glycerol concentration for 1, 2, 3, 4 days using Lactobacillus strain. Increasing in reaction time, remarkable increase in yield of 1,3-PDO was observed. During the bioconversion process, yield of 1,3-PDO was increased at certain time period as shown in TABLE. After 3 days of bioconversion process, constant yield was observed. So optimum yield was observed during 3 days of bioconversion of glycerol.

TABLE 3. **1,3-PDO yield at different temperature.**

Sr. No.	**Days**		**Yield(mol/mol)**			
		20˚C	**25˚C**	**30˚C**	**35˚C**	**40˚C**
1.	1	0.127	0.165	0.253	0.297	0.059
2.	2	0.142	0.193	0.285	0.345	0.083
3.	3	0.165	0.258	0.318	0.590	0.107
4.	4	0.165	0.258	0.319	0.590	0.125

Gas chromatography

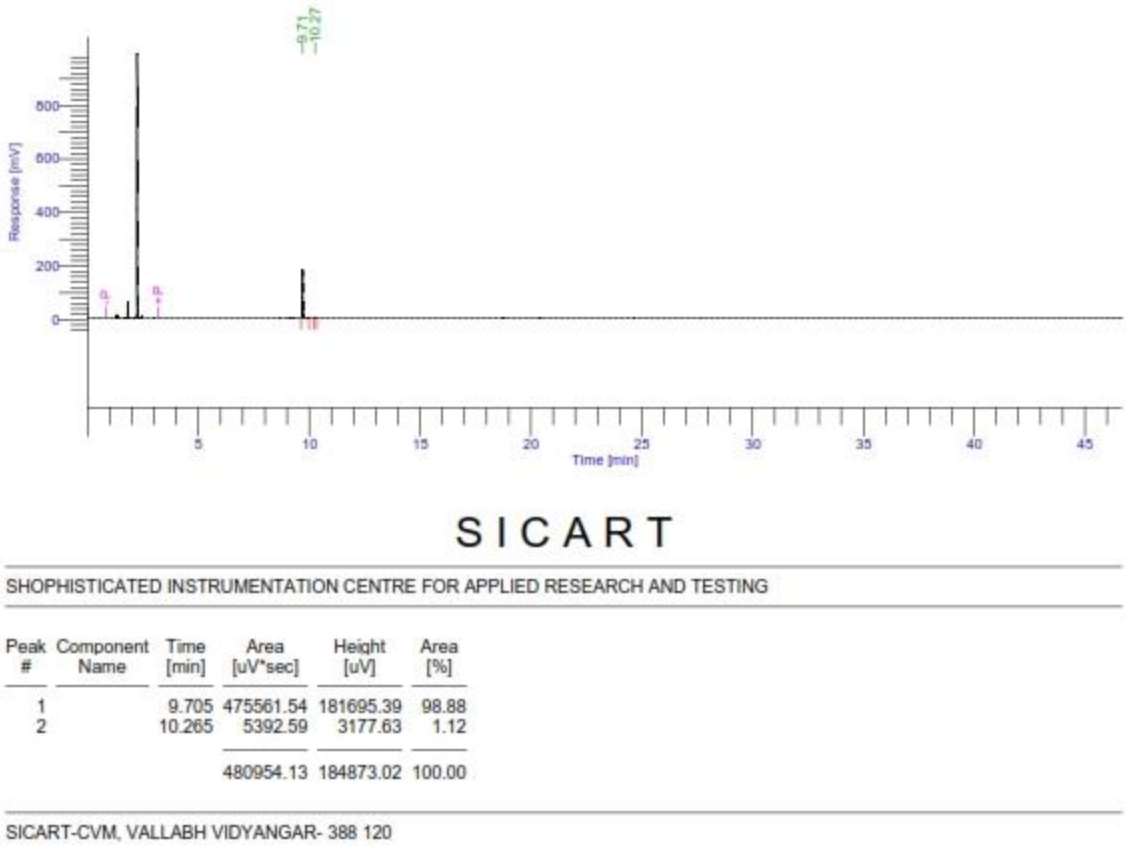

FIG. 1. **GC of 1,3-PDO.**

The chromatograph is shown in FIG. 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 9.705 min, 1,3-PDO was observed which was 98.88% pure. While at 10.265 min impurity was observed present in sample then no peak was observed till 45 mins.

FTIR spectroscopy

The FTIR spectrum of 1,3-PDO obtained from bioconversion of glycerol using Lactobacillus strain, is shown in FIG. 2. The structure of the 1, 3-PDO contains primary alcohol and alkane substitution. The two band observed at 2938.52 cm-1 and 2885.54cm-1 due to C—H streching frequency. The medium vibration observed at 1653.94 cm^{-1} which confirms the present of C—C—C streching. The —CH2— bending observed at 1474.84cm-1. Due to primary alcohol strong O—H bending vibration and C—O streching vibration observed at 1062.29cm-1 and 1233.71cm^{-1} respectively. So the presence of above describe functional group from FTIR spectra confirms the 1,3-PDO.

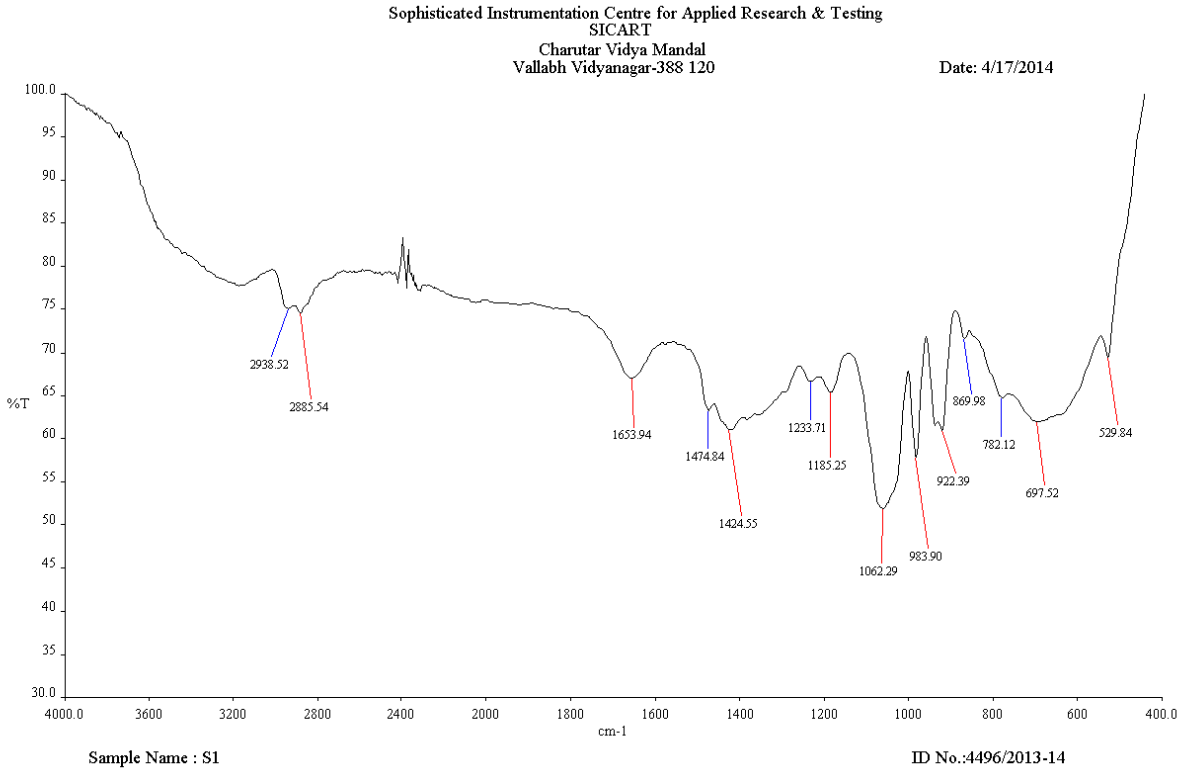

FIG. 2. **FTIR of 1,3-PDO.**

1H NMR spectroscopy

The 1H NMR spectrum of 1,3-PDO is shown in FIG. 3. 1,3-propanediol structure contains primary alcohol and methylene group. Multiplate was observed at 1.818 ppm which indicates the presence of –CH2– group. Singlet was observed at 2.555 ppm which proof the presence of –OH alcohol group in the sample. While triplet was observed at 3.868 due to –CH2 which attached to the alcohol in the sample. So according to the above 1H NMR spectra confirms the 1,3-PDO structure.

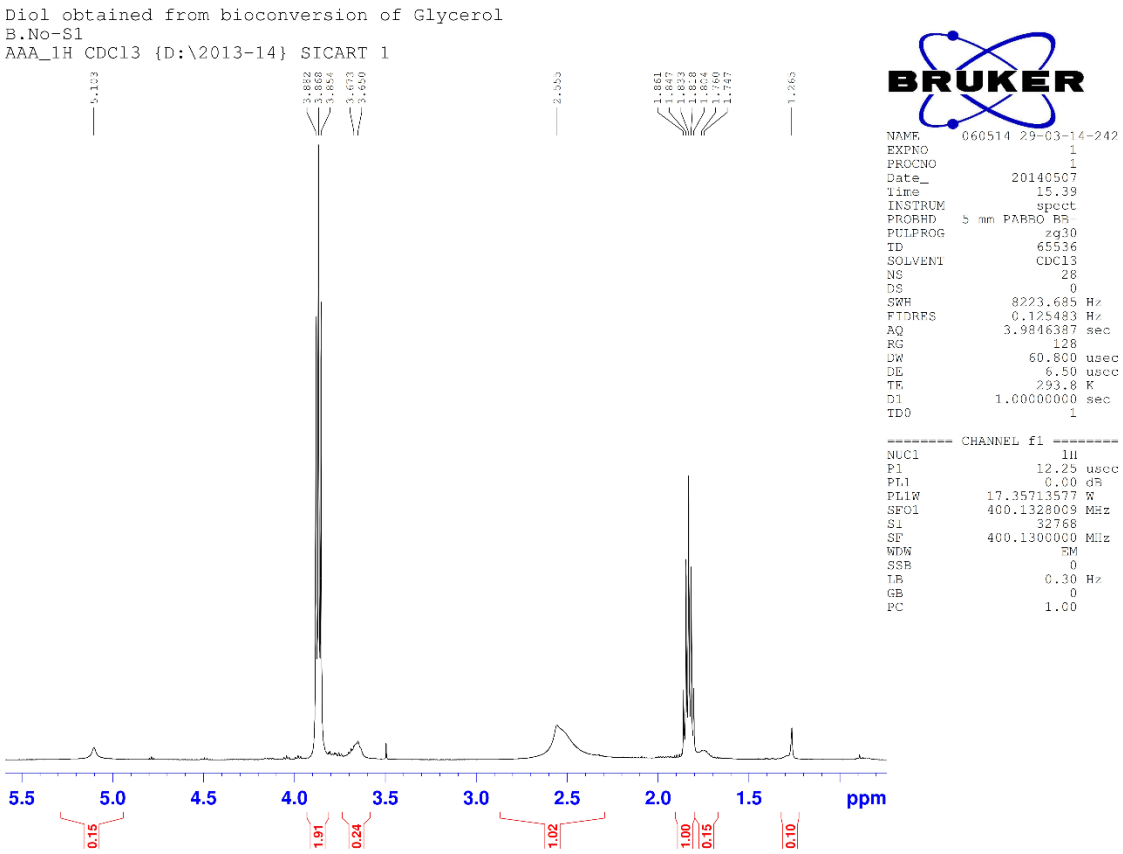

FIG. 3. **1HNMR of 1,3-PDO.**

Conclusion

By-product of biodiesel production i.e. glycerol was utilized in the biotechnology as carbon source for the production of specialty chemical 1,3-PDO using Lactobacillus strain. This helpful for economic production of biodiesel as its on high demand as well glut of crude glycerol was generated along with. The best 1,3-PDO yield were obtained in 3days process with at 35˚C temperature. 1,3-PDO can be utilized in the manufacturing of polyester polyol which can be utilized along with various isocyanates for the coating applications.

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